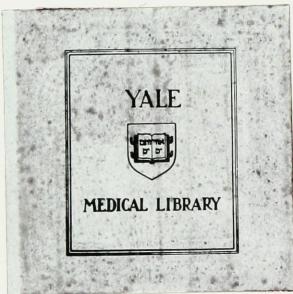


ATTENUATION OF EXERCISE-INDUCED BRONCHOSPASM
BY ASCORBIC ACID

ALAN SCHLESINGER

1980



ATTENUATION OF EXERCISE-INDUCED BRONCHOSPASM

BY ASCORBIC ACID

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DEDICATION

TO PAULA

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I. INTRODUCTION

A. Asthma

The classification of asthma has long been debated because a clear understanding of this disease has eluded researchers. The simplistic dichotomous division of all asthma patients into extrinsic and intrinsic at first appears attractive (patients who develop wheezing when exposed to a specific allergen and who have an "atopic" history being labelled extrinsic asthmatics, and those who have no history of allergies and whose wheezing is usually precipitated by a respiratory tract infection being referred to as intrinsic asthmatics). However, as new and unique precipitants of asthma continue to be discovered, these simple definitions fail to give full insight into the mechanisms involved in this complex disease.

A more recent approach to understanding and classifying asthma has been to pursue those humoral and neural mechanisms felt to be common to all forms of asthma. This approach may prove particularly useful in designing better treatment regimens. In this study we chose to investigate the mechanisms of asthma by focusing on a particular stimulus of bronchospasm: exercise.

B. Exercise-Induced Bronchospasm (EIB)

As early as the 17th century, Willis¹ observed the correlation between exercise and asthma:

"Whatsoever therefore makes the blood boyle or raises it into effervescence as violent motion of the body or mind, excess of extern cold or heat, the drinking of Wine, Vinery, yea sometimes meer heat of the bed doth cause asthmatical assaults to such as are predisposed."

Because exercise is a common, relatively predictable, and objectively measureable stimulus of asthma, researchers came to appreciate "the value of exercise as a diagnostic aid and a useful method for the assessment

of drugs in asthmatic subjects.² Therefore, EIB has now become a model for the study of asthma in general. Despite the prevalence and importance of exercise-induced bronchospasm, much remains unknown concerning its mechanisms. Various theories of the triggering stimulus for EIB have been proposed in the past, such as hypocapnia, increased minute ventilation, acidosis, stimulation of receptors in the pharynx or carotid body, and airway cooling. This confusing area has been partially elucidated by the development of the "heat exchange" theory,³ which correlates the total heat lost from the bronchial airways during exercise and the subsequent bronchospasm. Despite a clearer understanding of the factors responsible for the triggering of EIB, the mechanism initiated by this stimulus remains far less clear.

Several mechanisms for EIB have been suggested, and convincing arguments for and against all of them have been provided. Imbalance of the adrenergic and cholinergic nervous systems has been proposed as a possible factor, and studies have suggested that increased cholinergic tone,⁴ β -adrenergic blockade,⁵ or an enhancement in α -adrenergic activity⁶ may help explain the etiology of EIB. However, it has been shown by other investigators that adrenergic and cholinergic blockage do not change responses to exercise in asthmatics.^{7,8,9} Therefore, controversy exists concerning involvement of the autonomic nervous system in EIB.

Various chemical mediators have been suggested as the primary etiologic factor in exercise-induced asthma. This theory has largely been supported by the fact that drugs such as sodium-cromoglycate, which inhibits mediator release from mast cells, inhibit EIB if given before, but not after, exercise.¹⁰ A second argument that EIB is dependent on the release of mediators is that

the severity of attacks of EIB decrease when exercise is repeated after short intervals, suggesting release of a stored chemical mediator whose synthesis requires a certain amount of time.¹¹ Although evidence has been offered in favor of histamine,^{12,13} prostaglandins,^{14,15,16} slow-reacting substance of anaphylaxis (SRS-A),^{17,18} and bradykinin,^{19,20} the exact nature of the responsible mediator(s) remains unclear. Therefore, we decided to proceed with our study of the possible mechanisms of asthma by narrowing our focus to potential chemical mediators of EIB.

C. Ascorbic Acid (Vitamin C)

Although indirect evidence currently suggests that EIB is caused by release or formation of an agent with bronchoconstrictor activity,²¹ direct evidence remains lacking, and investigators have continued in their endeavors to better define the "mediator(s)" responsible. One approach has been to search for substances that potentially inhibit or counteract a suspected mediator of EIB and to determine whether these agents have a protective effect against bronchospasm initiated by exercise. One such agent, ascorbic acid, has long been considered a potential inhibitor of asthma.

As early as 1938, H.B.Hunt stated that "there is a certain amount of evidence that ascorbic acid may play a part in allergic conditions in general and asthma in particular."²² In addition, vitamin C has been found to have a role in the prevention of anaphylaxis and allergy^{23,24} and in the modulation of sensitivity of smooth muscle to spasmogenic agents.^{25,26,27} Zuskin was able to demonstrate an inhibition of bronchoconstriction by ascorbic acid by measuring the degree of bronchospasm induced in healthy subjects by histamine inhalation after either placebo or ascorbic acid.²⁸

Although this finding has been challenged,^{29,30} it does offer the possibility that vitamin C may have an anti-bronchospastic property.

Many theories have been advanced to explain the relationship between vitamin C and the reactivity of smooth muscle in general and bronchial smooth muscle in particular. A direct action of ascorbic acid on smooth muscle cells, perhaps mediated by a change in oxidation-reduction potential, has been suggested by some investigators,^{27,31} while others stress the importance of vitamin C in histamine metabolism.³² A third theory invokes fluctuations in prostaglandin levels (increase in PGE and/or decrease in PGF) induced by ascorbic acid.^{33,34} Finally, alterations in levels of cyclic nucleotides provide a potential explanation for the mechanism of ascorbic acid's effect on airway dynamics.³⁵

D. Present Work

Further elucidation of the mechanism by which the trigger of EIB leads to changes in airway resistance has practical and theoretical importance for a clearer understanding of EIB and asthma in general. The previously demonstrated anti-bronchospastic properties of vitamin C, as well as its involvement in the metabolism and activity of several potential mediators of EIB, suggested that investigation of this agent might prove fruitful. Since we believed that a chemical mediator etiology for EIB was correct, we hypothesized that ascorbic acid would offer protection to patients with EIB. Demonstration of a protective effect of vitamin C against EIB would not only lend support to the chemical mediator theory for the mechanism of EIB, but would direct attention toward certain specific members of the large group of mediators being considered.

Therefore, in a double-blind random fashion, we proposed to study

the protective effect of ascorbic acid vs. placebo against EIB in patients with documented, yet mild, exercise-induced asthma.

II. MATERIAL & METHODS

A. Subjects

Eleven asthmatic* subjects (4 male, 7 female) and ten healthy subjects (8 male, 2 female) without history of wheezing or asthma were recruited and informed consent was obtained. In order to select asthmatics with mild disease, members of the university and hospital community were chosen, avoiding the often more severe asthma of hospitalized and clinic patients. None of our subjects had been hospitalized for their asthma and none required corticosteroids. Each subject completed a detailed questionnaire concerning the presence of respiratory symptoms, allergies, and exercise-induced bronchospasm (EIB). The subjects' anthropometric data and responses to the questionnaire appear in Tables 1 and 3. All eleven asthmatic subjects complained of wheezing or dyspnea during or after exercise, while none of the ten healthy subjects had similar complaints.

B. Pulmonary Function

Using maximal and partial expiratory flow-volume curves (MEFV, PEFV) (see below), forced expiratory flow rates and volumes were measured in all subjects. A pneumotachograph-integrator system was used to obtain flow and volume, and the curves were recorded on a Brush 500 X-Y recorder (slew rate of 40 inches per second). After inspiring to 60-70% of vital capacity, the subjects exhaled forcefully to residual volume (creating the PEFV curve). They next immediately inspired to total lung capacity and performed a second forceful expiration to residual volume (creating the MEFV curve) (see Figure 2).

*asthma is a disease characterized by an increased responsiveness of the trachea and bronchi to various stimuli and manifested by a widespread narrowing of the airways that changes in severity either spontaneously or as a result of therapy (defined by American Thoracic Society)³⁶

From these curves were obtained forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), peak expiratory flow rate (PEFR), and flow rates measured at 60% of the baseline vital capacity below total lung capacity on maximal and partial flow volume curves (MEF40% and MEF40%(P), respectively). In addition, the flow rate after expiration of 50% of the tidal volume following a maximal inspiration (VMax50%) was measured for the baseline curves. Baseline pulmonary function data for all subjects appears in Tables 2 and 4.

C. Exercise Testing

All subjects performed the exercise studies on a cycloergometer (Monark) and cardiac frequency was measured with an electrocardiograph (Hewlett-Packard). Baseline heart rate was obtained and exercise was begun at a constant speed of 20 kilometers per hour against zero workload. At the end of each one minute interval, cardiac frequency was measured and the workload was increased by 150 kilopond meters per minute, keeping the pedalling speed constant throughout the experiment. Continued exercise against progressively larger workloads was maintained until either the heart rate reached 170 or the subject fatigued.

D. Experimental Design

a. Asthmatic subjects

1. First Day

All eleven asthmatics were instructed to take no medications for at least eight hours prior to each phase of the experiment. They were similarly instructed to refrain from any food or beverage containing large amounts of methyl xanthines (coffee, tea, cocoa, soft drinks, etc.) or ascorbic acid

(citrus fruits, juices, etc.). On the initial screening day, baseline PFT's were obtained by having the subjects perform the partial and maximal flow volume maneuvers three times, at one minute intervals, generating three pairs of partial and maximal flow-volume curves (baseline curves). Exercise was then performed as described above. After completion of exercise, three new pairs of MEFV and PEFV curves were obtained at one minute intervals (immediate post-exercise curves). A third set of three pairs of curves was generated five minutes after termination of exercise (five-minute post-exercise curves). Two metered doses (0.65 mg each) of metaproterenol sulfate (Alupent^R) inhaler were then administered, and ten minutes later three final pairs of MEFV and PEFV curves were obtained (post-alupent curves) (see Figure 1) FVC, FEV₁, PEFR, MEF40%, and MEF40%(P) values were determined for the four sets of curves by averaging the values obtained from the three pairs of MEFV and PEFV curves in each set. Those subjects who demonstrated sufficient EIB (20% reduction in MEF40% and MEF40%(P) after exercise) were allowed to proceed to the remaining phases of the experiment.

2. Second and Third Days

On the second and third days, each subject ingested (in double blind, random order) vitamin C (ascorbic acid 500mg) and placebo (sucrose) one hour prior to repeating the first day's experiment. In identical fashion, PFT's were measured before exercise, after exercise, and after alupent.

b. Healthy Subjects

Because our asthmatic subjects provided an internal control (taking vitamin C on one day and placebo on the other), control subjects were required only for the initial screening day without drugs to demonstrate that the asthmatics' response to exercise differed from that of normal subjects.

III. RESULTS

A. Pulmonary Function Response to Exercise Without Drugs

During the initial screening day for the asthmatics and the single experimental day for the healthy subjects, data measuring pulmonary function changes in response to exercise were collected. Neither group received vitamin C or placebo during this phase of the experiment in order to allow an unbiased determination of the presence or absence of EIB. Tables 5 through 9 display the changes in FVC, FEV_{1.0}, PEFR, MEF40% and MEF40% (P) after exercise for the asthmatic subjects, listing both the absolute quantities and the values as a percent of the before-exercise, or baseline, measurements (% baseline). In a similar fashion, Tables 10 through 14 represent the responses of the healthy subjects. Figures 5 through 9 graphically demonstrate these data. All changes from baseline were tested for statistical significance using the Student's paired t-test.³⁷

In the asthmatic group, all five parameters demonstrated the typical response for patients with EIB: bronchodilation during exercise (and therefore, immediately post-exercise), bronchoconstriction commencing within five minutes after exercise, and a marked bronchodilation after inhalation of metaproterenol sulfate (post-alupent) (see Figures 3 and 4). The post-exercise bronchoconstriction is the most characteristic response to exercise in patients with EIB, and this change from baseline was statistically significant ($p < .01$) for all five parameters measured. Although none of the parameters revealed a statistically significant change from baseline immediately post-exercise, there was a consistent trend toward bronchodilation. After administration of alupent, the bronchodilation experienced by the asthmatics was statistically different from baseline ($p < .05$) for all parameters except FVC.

In contrast, healthy subjects revealed no post-exercise bronchoconstriction except for a small and statistically insignificant decrease in FVC. All other changes of pulmonary function in response to exercise

in non-asthmatics were not found to be significant when analyzed statistically except for an increase in MEF40% ($p < .05$) five minutes post-exercise (bronchodilation) and increases after alupent in MEF40% ($p < .01$) and MEF40% (P) ($p < .01$).

Therefore, the asthmatic subjects as a group demonstrated significant bronchospasm post-exercise that was not observed in normal controls, confirming the diagnosis of EIB. Both groups revealed bronchodilation in response to alupent (asthmatics > healthy subjects).

B. Pulmonary Function Response to Exercise: Placebo

Tables 15 through 22 display the measured change in pulmonary function with exercise after placebo and without drugs both as absolute quantities and as a percent change from baseline before exercise (" Δ PFT"). This information is represented graphically in figures 6 through 10. Tables 23 through 25 compare the differences in these Δ PFT's between placebo and without drugs ($\Delta (\Delta PFT)$). These differences between the Δ PFT's after placebo vs. without drugs was not within statistical significance for all five parameters. Therefore, no placebo effect was found. It is of interest that the changes in FVC, FEV₁, and PEFR demonstrated a trend of increased bronchospasm after exercise with placebo as opposed to the initial screening day. Similarly, there was less bronchodilation in the asthmatic subjects immediately post-exercise and after alupent, as measured by all five variables, when exercise followed ingestion of a placebo as opposed to without drugs. However, this difference was not within statistical significance.

C. Pulmonary Function Response to Exercise: Vitamin C

Tables 26 through 33 display the change in PFT's with exercise following

ingestion of vitamin C vs. placebo both in the absolute and the Δ PFT form. This comparison is graphically portrayed in Figures 15 through 19. Tables 34-36 contain the $\Delta(\Delta$ PFT) measurements for vitamin C vs. placebo. It is of note that ingestion of vitamin C, when compared to placebo, led to a lesser degree of bronchospasm after exercise as measured by all five parameters (statistically significant for FVC ($p < .05$) and $FEV_{1.0}$ ($p < .05$)). Similarly, vitamin C was associated with an increase in bronchodilatation relative to placebo both immediately after exercise and post-alupent as measured by all five variables. These differences between vitamin C and placebo were statistically significant for the immediate post-exercise measurements of PEFR ($p < .05$) and MEF40% (P) ($p < .05$) and for the post-alupent values of all parameters ($p < .05$) except MEF40%(P). Therefore, vitamin C, relative to placebo, was found to cause significant decrease in bronchospasm after exercise (for FVC and $FEV_{1.0}$) in patients with EIB. In addition, vitamin C led to a significant enhancement of bronchodilatation both immediately post-exercise and after alupent as demonstrated by several of the parameters we used to measure pulmonary function.

Table 37 contains a summary of the paired t-test results for the comparisons of the efficacy of placebo vs. no drug and vitamin C vs. placebo.

In order to demonstrate the improvement in individual subjects' PFT's with AA, Figure 20 compares the change in FEV_1 with exercise for all twelve subjects after vitamin C and after placebo. Although there is some overlap of the response to exercise on the two experimental days, the measurements demonstrate the general tendency toward increased bronchospasm after placebo relative to vitamin C.

D. Analysis of Variance

In order to demonstrate that there was no variation in measurements of baseline pulmonary function between the various experimental days, a one-way analysis of variance was performed for the baseline FVC, FEV_{1.0}, PEFR, MEF40%, MEF40%(P), and Vmax50% on the vitamin C, placebo, and no-drug days. The results, which appear in Table 38, confirm that there was no difference in pulmonary function before exercise between the three experimental days.

IV. LITERATURE REVIEW: ASTHMA

A. History³⁸

Hippocrates (460-370 BC) made the first known reference to asthma but did not discuss the disease in detail. Galen's understanding of this disease was confused by his anatomical misconceptions. He believed that conduits connected the brain and the respiratory tract. He considered asthma to be a mechanical interference of respiration caused by the descent of secretions from the brain into the respiratory tract. It was not until the Renaissance that Galen's dogma was discarded and more precise observations and descriptions followed. Concepts of etiology, however, remained confused. Laennec's treatise on auscultation in 1819 lead to great advances in the understanding of all pulmonary disease. He suggested several precipitating factors of asthmatic paroxysms but felt that the final common etiologic event was a temporary alteration in nervous function. He believed that contraction of smooth muscle fibers surrounding the bronchi and air vescicles provided a physiological explanation for the physical findings observed.

Our understanding of asthma has obviously advanced greatly since these beginnings as we have a far clearer picture of anatomy and pathophysiology. However, the mechanisms responsible for producing the clinical symptoms have remained a subject of debate, and this confusion is illustrated by the controversies concerning the classification of asthma.

B. Classification of Asthma

Some researchers choose to separate all asthmatics into two large groups: extrinsic or intrinsic. Extrinsic bronchial asthma, or allergic asthma, is a disease predominantly of children and young adults. Episodes

of bronchospasm are short-lived, interspersed between symptom-free intervals, and temporally related to exposure to certain causitive antigens. These patients often have an "atopic" history, demonstrating other allergic phenomena such as eczema or hay fever. Allergic asthmatics generally respond well to medical treatment, but they may have recurrences in later adult life.

Intrinsic asthmatics are a more heterogeneous group of patients in whom antigenic exposure does not appear to play a role in producing bronchospasm. These patients tend to be adults over thirty-five years of age, and they usually have no history of allergies. Because respiratory tract infections seem to be important in the development of symptoms, this form of asthma has also been referred to as infective asthma. Aside from increased cough and sputum production, these bronchospastic episodes closely resemble those of allergic asthmatics. Infective asthma is more likely to be a chronic problem that is less responsive to medical management.

Despite the obvious convenience of creating the categories of extrinsic and intrinsic asthma, this strict dichotomy breaks down with closer analysis. Many patients are labeled mixed asthmatics, demonstrating characteristics of both groups; they are atopic and have an allergic basis to their bronchospasm, but their acute episodes are usually preceded by a respiratory tract infection. The concept that a large group of asthmatics develop symptoms on a non-allergic basis has itself been challenged, and some have argued that immunological factors are involved in all forms of asthma even though they cannot be demonstrated. Others have proposed that the cases of bronchospasm in which immunological involvement cannot be implicated should not be considered asthma.

Further problems with this simplistic dichotomy become evident on more careful scrutiny of asthmatic patients. Many have been classified into minor categories of asthma, but these groups tend to cross the artificial boundary between intrinsic and extrinsic asthmatics. One such group, exercise-induced asthma, contains a diverse population of asthmatics from those with a strong history of atopy to others who have classic intrinsic bronchospasm.

There are other subtypes of asthma that create similar problems in classification.³⁹ Patients with "asthmatic-bronchitis" may be thought of as having chronic bronchitis with superimposed symptoms of asthma without a clearly defined allergic basis. A small number of patients with intrinsic asthma manifest the triad of asthma, aspirin sensitivity, and nasal polyposis. Their bronchospasm is usually preceded by aspirin ingestion. A final group of patients, dual Type I and Type III reactors, develop an acute attack of bronchospasm (IgE-mediated) within ten to fifteen minutes after exposure to an allergen and then a second (IgG-mediated) response in two to six hours.

Confused by these overlapping artificial categories, some researchers began to consider asthmatic patients as a spectrum from those with a strong history of allergic stimulation to those with no known immunologic basis for their bronchospasm. Although each patient is particularly susceptible to certain stimuli, there may be some precipitants of asthma that provoke an attack in all patients. Therefore, what is needed for the study of asthma is a suitable experimental human model in which acute exacerbations can be induced.

V. LITERATURE REVIEW: EIB

A. History

After Willis first recognized in 1684 that asthma could be stimulated by exercise (or "whatsoever...makes the blood boyle"), Floyer recorded in 1717 an attack of asthma precipitated by exercise and stated that "all violent exercise makes the asthmatic to breathe short."⁴⁰ He further noted that different types of exercise varied in their ability to provoke wheezing.

Very few references were made to this correlation between exercise and asthma throughout the eighteenth and nineteenth centuries. In 1946 Herxheimer measured the change in vital capacity after exercise in asthmatic subjects,⁴¹ performing the first objective study of the relationship of asthma to exercise. He concluded that hyperventilation was the cause of this bronchospasm stimulated by exercise.

Herxheimer's work stimulated many researchers interested in this field, but there was much confusion due to conflicting reports of either increased bronchospasm or improvement of pulmonary function after exercise. Jones et al in 1962 first attempted to explain this paradox and studied the response of asthmatic children to running exercise for brief (two minute) and prolonged (eight to twelve minutes) periods. They found that the FEV₁ increased after the former but fell markedly after longer durations of exercise.⁴² Although his findings were not supported totally by later work, he did demonstrate the importance of the duration of exercise.

Further work in this field suffered from several misconceptions concerning the stimulus for EIB. Various conflicting theories for the triggering stimulus were proposed, and different forms of exercise were felt

to have varying ability in promoting bronchospasm. It was not until recently, when McFadden et al developed the heat exchange theory for the trigger of EIB (see below), that much of the conflicting data could be explained and a clearer understanding of this disease became possible.

B. EIB as a Model for Asthma

Jones et al first suggested that EIB might be used as a diagnostic test for asthma in children based on his demonstration that nineteen of twenty-one asthmatic children developed bronchospasm (measuring FEV₁) after eight to twelve minutes of running.⁴³ Much research followed to confirm that exercise was a reliable stimulus for bronchospasm in asthmatics. Godfrey demonstrated that ninety percent of asthmatic children had a ten percent decrease in PEFR after exercise⁴⁴ whereas this response is rarely seen in healthy children.⁴⁵ Using a fifteen to twenty percent decrement in FEV₁ as a criterion, others have demonstrated a sixty to seventy percent incidence of post-exertional asthma among children.^{46,47}

Similar results have been observed in adults. McNeill et al demonstrated a thirty to seventy-five percent decrease in FEV₁ after exercise in all ten adolescents and young adults he studied.⁴⁸ A meticulous study by Haynes, Ingram, and McFadden measured airway resistance (using a constant volume plethysmograph), airway specific conductance, FEV₁, flow rates between twenty-five and seventy-five percent of FVC (MMFR), and maximal flow rates at isovolumes between forty and sixty percent of TLC ($\dot{V}_{\text{max iso}}$) in twenty-one asthmatics and eight normal controls. They found that all of the asthmatics, in contrast to the controls, had a significant response that was measureable by at least one variable regardless of the severity of their clinical history.⁴⁹

Finally, the development of the PEFV curve, combined with instantaneous measurements of flow rates at low lung volumes, provided evidence that exercise was even more of a ubiquitous stimulus for bronchospasm than had previously been comprehended (see below).⁵⁰

Therefore, the ability of exercise to stimulate an attack of bronchospasm in most, if not all, asthmatics has led investigators to consider EIB as a suitable model for the diagnosis and study of asthma.^{51,52,53,54} Jones has stated that "a post-exercise fall in FEV_1 is so constant in the asthmatic that a failure to demonstrate it should lead to reconsideration of the diagnosis or of the technique of the test."⁴² Some have suggested that EIB may not be an isolated disease, but rather that exercise is just one means of stimulating bronchospasm in asthmatics.⁵⁵

Exercise has been found to have several advantages compared to other methods of evoking an acute asthmatic response. Unlike the inhalation of pharmacologic or immunologic agents, exercise avoids direct stimulation of the airway itself and artifactual alterations in pulmonary function.^{49,51} In addition, exercise produces a response in atopic as well as non-atopic asthmatics.⁵⁶ Finally, exercise can be used in the laboratory to safely and reliably induce exacerbations of bronchospasm.⁴⁹

C. Response to Exercise in Asthmatics and Healthy Subjects

The most accurate and sensitive parameters for measuring the response of asthmatics to exercise has long been the subject of debate. Researchers have commonly used FEV_1 ,⁴² PEFR,⁵⁷ airway resistance,⁴⁹ maximal mid-expiratory flow rates (MMFR),⁴⁹ total lung capacity (TLC),⁵³ and FVC⁵³ to detect changes in pulmonary function. The typical response of asthmatics to five to

ten minutes of exercise is an improvement in pulmonary dynamics during exercise and a marked decrease commencing three to five minutes afterwards.²

The response of healthy subjects has been reported to be either a statistically insignificant change from baseline⁴³ or else markedly less than the change observed in asthmatics.²

The measured improvement in pulmonary function during exercise in asthmatics, which has been attributed to the release of endogenous catecholamines,⁵⁵ has been exploited in attempts to develop more sensitive measures of the response to exercise. Jones suggested a "bronchial lability index" $\frac{\text{Fall of FEV}_1 + \text{Rise of FEV}_1}{\text{Predicted Normal FEV}_1 \text{ at rest}} \times 100$, where "Fall in FEV₁" is the maximal decrease after five to eight minutes of exercise, and "Rise in FEV₁" is the maximal bronchodilatation after isopenaline aerosol and one minute's exercise. Studying asthmatic and healthy children, he demonstrated that, with few exceptions, the lability index was greater than twenty percent in asthmatics and less than fifteen percent in control subjects.⁵⁸

Bouhuys questioned the traditional use of the MEFV curve to assess subtle changes of induced bronchoconstriction because it had been demonstrated previously that a deep inspiration might lead to a transient increase in airway resistance.^{59,60} He demonstrated that flow rates on PEFV curves, where the maneuver is initiated by a less than complete inspiration, were more sensitive in detecting bronchoconstriction than flow rates on MEFV curves at comparable volumes.⁶¹ He postulated that this might reflect inhibition of bronchoconstriction that is usually produced by the deep inspiration involved in the MEFV maneuver and potentially obscures subtle mechanical changes. He further criticized the use of standard measurements of pulmonary

function (FEV₁, MMFR, PEFR, etc.) because they are partially a function of the effort-dependent (early) part of the FVC. He advocated the use of instantaneous flow rates at lower lung volumes (MEF40%, MEF40% (P))⁶², using electronic circuits to record flow and volume⁶³, in order to more accurately measure the state of the small airways during the effort-independent (late) portion of the FVC. These techniques led to increased sensitivity in measuring bronchospasm initiated by exercise in asthmatics⁵⁰ and by pharmacologic agents in healthy subjects.⁶⁴

D. Trigger of EIB

1. Early concepts

In attempting to elucidate the "trigger" that is activated by exercise, researchers have accumulated conflicting data. Furthermore, different forms of exercise have been shown to result in varying degrees of bronchospasm, a finding that has generated added debate.

a. Hypocapnia has long been considered a potential cause of bronchoconstriction. Hafez and Crompton⁶⁵ demonstrated a small but statistically significant decrease in FEV₁ in asthmatics after hyperventilation that was not observed in bronchitics. Furthermore, there was no change in pulmonary function after hyperventilation induced by carbon dioxide inhalation. Furguson et al measured P CO₂ levels after exercise in healthy controls and in patients with EIB and diffuse lung disease. Three minutes after exercise, the P CO₂ of the asthmatics was 23 mm Hg compared with 37 for controls and 31 for patients with diffuse lung disease. They postulated that the hypocapnia produced bronchospasm because it could be reversed by adding carbon dioxide to the inspired air.⁶⁶ In addition, Fisher et al

demonstrated that bronchospasm stimulated by running could be reversed by inhaling six to eight percent carbon dioxide but not by inhaling atropine sulfate, suggesting an effect of carbon dioxide on smooth muscle unrelated to a vagal efferent reflex pathway.⁵⁴

b. Others have claimed that mechanical hyperventilation itself, and not hypocapnia, is the inciting stimulus for EIB. Chan-Yeung demonstrated bronchoconstriction in asthmatic patients despite normal or slightly increased concentrations of carbon dioxide in airways and blood.⁹

c. A third popular theory for the trigger mechanism is the release of lactic acid from skeletal muscle cells during the anaerobic phase of exercise. Many researchers have correlated bronchospasm with the presence of metabolic acidosis and/or increased circulating levels of lactate after exercise in patients with EIB.^{9,54,67} Schiffman et al postulated that this change in lactic acid concentration might be causing bronchospasm by stimulating the carotid bodies, and he demonstrated that breathing one hundred percent oxygen attenuated EIB in patients with intact carotid bodies but had no effect in asthmatics who had undergone bilateral carotid body resections.⁶⁸ Other suggested pathways for lactate's effect include release of mediators from mast cells or increased production of bradykinin caused by elevated hydrogen ion concentration.³

2. Respiratory Heat Exchange

Different responses have been noted in asthmatics when exercise has been accompanied by nasal breathing as opposed to oral breathing. Souhrada et al postulated that this might reflect the presence of receptors

in the oropharynx sensitive to temperature or humidity. These receptors would be stimulated by exposure to cold, dry air breathed through the mouth but not by air that had been "conditioned" as it was warmed and humidified during its passage through the nasal airways.⁶⁹ Further evidence for this theory was provided by studies demonstrating that EIB can be attenuated by oral anaesthetics, suggesting that EIB might be triggered by a reflex mechanism initiated by stimulation of these irritant receptors.⁷⁰

The specific type of exercise used to elicit EIB has long been known to be an important determinant of the degree of bronchospasm produced. Fitch and Morton showed that EIB could be induced in forty asthmatic subjects after eight minutes of exercise in 72.5 percent of running tests, 65 percent of cycling tests, and 35 percent of swimming tests.⁷¹ Others have agreed that running is a more potent stimulus than cycling, and that walking and swimming have variable effects.⁷²

Much of the conflicting data presented above was put into perspective by a series of ingenious experiments by McFadden et al. Addressing first the question of different responses to different forms of exercise, they demonstrated that airway obstruction was dependent on the amount of stress of participating muscles rather than on the total amount of external work.⁷³ Larger workloads in relation to muscle mass stimulated higher minute ventilation, higher hydrogen ion concentration, lower end-tidal carbon dioxide tension, and increased bronchospasm.

Approaching the effects of hyperventilation and hypocapnia, they simulated minute ventilations achieved during exercise using a partial rebreathing technique with variable end-tidal carbon dioxide tensions.

They found that even the most marked changes induced by hyperventilation or hypocapnia were small relative to bronchospasm produced by exercise at higher end-tidal carbon dioxide levels. This led to the conclusion that hypocapnia and the mechanical effects of bulk air flow were not the stimulus for EIB.⁷⁴

The effect of lactic acid and hydrogen ion concentration were then addressed. Patients with EIB were exercised until bronchospasm was achieved. This experiment was repeated with sodium bicarbonate infusions to maintain baseline arterial pH. No change in the degree of bronchospasm was observed. Resting subjects with EIB received infusions of lactic acid at levels higher than those produced endogenously during exercise with no measureable change in pulmonary function. They concluded from these experiments that neither pH nor lactate levels played a key role in triggering bronchospasm.⁷⁵

McFadden et al then observed that asthmatic subjects had greater decreases in pulmonary function when they exercised in a cold environment.⁷⁶ Because atropine did not decrease cold air's stimulus to bronchospasm, stimulation of sensory nerve endings and vagal reflexes did not appear to play a role.⁷⁷ Esophageal temperatures at the level of the mid-trachea were shown to be decreased relative to behind the heart and in the rectum when exercise was performed in room air, and this change in temperature could be increased by breathing colder and drier air.⁷⁸ Therefore, it appeared that bronchoconstriction was due to a local effect of incompletely conditioned air reaching the lower respiratory tract.

As a final series of experiments, McFadden et al studied the effects of heat and humidity on EIB by exercising asthmatics breathing, in random

order, air at four conditions: ambient room temperature and humidity, body temperature and ambient humidity, ambient temperature and one hundred percent humidity, and body temperature and one hundred percent humidity. Asthmatics breathing ambient air had typical bronchospasm, and heating the air led to no improvement. Some reduction in airway obstruction was achieved by humidifying the air, while saturated air at body temperature prevented EIB.⁷⁹ McFadden et al suggested that asthmatics may not be effectively conditioning air or may be unusually sensitive to airway cooling.³

The heat-flux hypothesis, the endpoint of this series of logical experiments, states that there is an association between the heat lost from the airways and the subsequent change in pulmonary function.⁸⁰ Air is warmed as it passes the respiratory tract mucosa moving from the environment to the lungs. As the temperature increases, the capacity of the air to hold moisture is enhanced and it is humidified by evaporation from the airway mucosa. This evaporation accounts for a large portion of the airway heat loss. Therefore, the higher the rate of ventilation or the colder and drier the air, the greater the heat exchange.³ To test this theory, McFadden et al had resting subjects with EIB inhale air of various temperatures and humidities to simulate the "thermal burden" of exercise.⁸¹ As expected, increased ventilation and decreased temperature or saturation of the air led to larger decrements in FEV₁. By plotting the percent change in FEV₁ (% ΔFEV₁) against the respiratory heat exchange (RHE) in Kcal/minute, the authors demonstrated a linear relationship between these two parameters: % ΔFEV₁ = 26.14 RHE - 4.16.³

E. Theories of the Mechanism of EIB (Beyond the Trigger)

Potential mechanisms of EIB, paralleling those of asthma in general, have stressed both chemical mediators and involvement of the autonomic nervous system.

1. Autonomic Nervous System (ANS)

Although a chemical mediator theory for EIB has received much attention recently, some still advocate ANS imbalance as a potential factor. The arguments proposed can be assigned to three main theories.

a. β -adrenergic Theory

β -adrenergic dysfunction has long been suggested as a mechanism of bronchoconstriction in asthmatics. Stimulation of β -adrenergic receptors was shown to cause bronchodilation by a direct effect on smooth muscle cells. This response has been associated with increases in cyclic AMP (cAMP) levels. Szentivanyi summarized previous evidence and formulated the β -adrenergic theory which stated that the mechanism for bronchospasm in asthmatics might be defective β -adrenergic receptors, possibly secondary to an enzymatic defect in adenylate cyclase.⁸² Parker and Smith demonstrated that leukocytes from asthmatics had a significant decrease in their cAMP response to β -adrenergic agents, suggesting some form of β -blockade.⁸³ This theory has been supported by the demonstration that pharmacologic β -blockade increases bronchospasm caused by allergens⁸⁴ or methacholine.⁸⁵ In addition, drugs which prevent breakdown of cAMP by inhibiting phosphodiesterase (i.e. methylxanthines) help to relieve asthma.⁸⁶

β -adrenergic dysfunction has also been suggested in EIB. Jones demonstrated increased bronchial lability after exercise in five of six asthmatics and in seven of forty-five controls with mild β -blockade induced by propranolol.⁸⁷ Patel et al suggested that β -blockade leads to increased α -adrenergic response to catecholamines released during exercise.⁵ Beil et al found that the increase in bronchospasm experienced by patients with EIB after propranolol could be inhibited by α -adrenergic blockade with phentolamine.⁶ Although β -sympathomimetics have been shown to improve symptoms of EIB, this cannot be rigorously assigned to a direct

smooth muscle effect, as they also prevent mast cell degranulation.

In addition, there is evidence that sympathomimetics' prevention of post-exertional bronchospasm may be distinct from their bronchodilating effect.⁸⁸

b. α -Adrenergic Theory

Bianco et al demonstrated the presence of α -adrenergic receptors in bronchial smooth muscle cells and observed that indoramin, an α -adrenergic blocking agent, was able to prevent EIB as measured by FEV₁ and airway conductance. He postulated that, after exercise, endogenously released catecholamines stimulate the α -receptors which may be either too numerous or too sensitive relative to the β -receptors in patients with EIB.⁸⁹ However, Seale et al repeated this work using PEFR to evaluate pulmonary function and found no attenuation of bronchospasm.⁹⁰ In addition, indoramin has been shown to have an antihistaminic effect which could account for its protective effect.⁸⁹

c. Cholinergic Theory

Parasympathetic reflexes have been shown to play an important role in airway dynamics. One example is the vagally mediated cough or "irritant" receptor reflex.⁴ These receptors can be stimulated by cough, rapid inspiration or expiration, histamine, citric acid, chemically inert dusts, or sulfur dioxide. In addition, it has been shown that immunologically induced mediator release can be stimulated by parasympathomimetic drugs.⁹¹

Kiviloog implicated vagal involvement in EIB when he found a positive correlation between post-exercise bronchospasm and methacholine sensitivity. He concluded that bronchoconstriction by both stimuli worked

through nonspecific bronchial hyperreactivity.⁹² Crompton reported a case of EIB that was prevented by atropine, suggesting that vagal reflexes might be involved.⁹³ Evidence to the contrary is provided by an experiment by Fischer et al in which atropine did not prevent EIB in seven asthmatic subjects.⁵⁴ Some researchers have suggested that cholinergic pathways cause bronchospasm in EIB when obstruction is limited to large airways, while mediators are involved in patients with more widespread bronchoconstriction.^{94,95} McFadden et al has postulated that cholinergic reflexes themselves may be stimulated by mediator release.⁹⁵

Sey et al performed an experiment which suggested that there is no ANS involvement in EIB. He measured the change in PEFR induced by exercise in ten asthmatic children after either α -adrenergic, β -adrenergic, or cholinergic blockade and found no change in the post-exercise decrement in pulmonary function.⁷

2. Mediators

The release of histamine and other chemical mediators from mast cells in antigen-induced, IgE-mediated asthma has stimulated researchers to look for a similar mechanism for EIB. Sodium cromoglycate is a drug which stabilizes mast cells by preventing cAMP breakdown. It has also been found to have phosphodiesterase inhibitory capacity. Silverman et al demonstrated that this agent inhibits EIB if given before, but not after, exercise, suggesting that release of pre-formed, stored mediators are involved.¹⁰ The protective effect of this drug has been confirmed by others.^{96,97,98,99,100,101} The presence of a refractory period when bronchospasm is repeatedly induced by exercise provides a second argument for the

mediator hypothesis.^{9,48,102}

a. Histamine

The release of histamine is closely linked to the β -adrenergic system. As is the case in smooth muscle cells, β -adrenergic stimulation leads to an increase in cAMP in mast cells which stabilizes their membranes and hence prevents degranulation. Evidence for histamine's involvement in asthma independent of the β -adrenergic nervous system stems from the demonstration that allergens, combining with IgE molecules on mast cell membranes, lead to degranulation and histamine release.¹⁰³ Furthermore, inhalation of histamine aerosol has been shown to produce bronchospasm in asthmatics, although some have suggested that this may represent stimulation of irritant receptors in patients with hyperactive airways. Nevertheless, subcutaneous histamine causes bronchospasm in asthmatics with doses smaller than those necessary to produce similar symptoms in healthy subjects.¹⁰⁴ These effects of histamine in asthmatics have lead to suggestions that histamine may play a central role in EIB.

Anderson et al demonstrated that β -sympathomimetics attenuate EIB through means other than bronchodilatation.⁸⁸ Zielinski et al found that thiazinamium, an antihistaminic agent, prevented EIB and suggested that prevention of histamine release might be another effect of β -adrenergic stimulation.¹⁰⁵ More direct evidence for involvement of histamine in post-exertional asthma was provided when Anderson et al demonstrated that arterial plasma histamine levels increased during exercise in patients with EIB, and this rise was diminished by β -sympathomimetics.¹⁰⁶ This finding contradicted earlier work demonstrating no increase in histamine turnover during exercise in these patients.¹⁰⁷

b. Slow-Reacting Substance of Anaphylaxis (SRS-A)

The chemical structure of this acidic lipid has not been completely characterized. As opposed to the brisk contraction of *in vitro* smooth muscle preparations induced by histamine, this substance causes a more gradual contraction. In addition, relaxation is a slower process when SRS-A is washed from the tissue baths as compared to other stimulants of muscle contraction. SRS-A does not appear to be stored in a preformed state in the tissues of man or the rat, and antigen-antibody reactions have been shown to be required for its release.¹⁰⁸

Involvement of SRS-A in asthma has been postulated as this chemical can be recovered from lung tissue that has been sensitized with IgE and then immunologically challenged. Because its level rises in concert with histamine, it is felt that SRS-A is also released from mast cells. In addition, SRS-A has been shown to potentiate the effect of histamine on smooth muscle.¹⁰⁸

Indirect evidence has been provided for involvement of SRS-A in EIB. Orange et al demonstrated that diethylcarbamazine citrate inhibits release of SRS-A in rats,¹⁰⁹ and Sly et al subsequently showed that this substance, given before exercise, prevented EIB in fifteen of twenty asthmatic children.¹¹⁰

c. Bradykinin

Kinins have been implicated in vasodilation, increased vascular permeability, smooth muscle contraction, pain, and possibly leukocyte margination, yet their mechanism of action remains unknown.¹¹¹

Kinins cause contraction of uterine and guinea pig ileal muscle *in vitro*. Because this effect cannot be prevented by atropine, cholinesterase

inhibitors, or ganglionic blocking agents, it is postulated that kinins have a direct action on smooth muscle. This effect on certain types of smooth muscle, including bronchial, is variable, causing relaxation in some systems and contraction in others.¹¹¹

The involvement of bradykinin in asthma was suggested by Collier et al with the demonstration that this agent produced bronchospasm in anesthetized guinea pigs and in isolated, perfused guinea pig lungs.¹¹² Herxheimer et al reproduced this work in guinea pigs and further showed that inhalation of bradykinin aerosol caused a decrease in vital capacity in asthmatics but not in healthy subjects.¹¹³ However, no experiments have been carried out to study the involvement of bradykinin in EIB.

d. Prostaglandins (PG)

Prostaglandins are twenty-carbon, unsaturated fatty acids composed of a cyclopentane ring and two aliphatic side chains. Although there are four main groups of PG's, PGE₂ and PGF₂^α have received the most attention in pulmonary medicine. Both are synthesized from arachidonic acid by prostaglandin synthetase. PGF₂^α is formed by reduction of the intermediate form, cyclic endoperoxide, whereas isomerization leads to production of PGE₂.

Although PG's are found in most mammalian tissues, the lung has one of the highest concentrations.¹¹⁴ Lung parenchyma contains ten to twenty times more PGF₂^α than PGE₂, while two to three times more PGE₂ than PGF₂^α can be found in isolated bronchi.¹¹⁴ PGE's have a bronchodilating effect in isolated human bronchial muscle which is not altered by atropine or antihistamines.¹¹⁵ PGF₂^α causes bronchoconstriction that is not modified by SRS-A, α - and β-adrenergic agents, or serotonin.¹¹⁴ Therefore, it appears that PG's influence bronchial smooth muscle tone independently of the ANS or other chemical mediators.

Some have suggested that PG's act directly on smooth muscle by raising cAMP levels. In human lung fragments and peripheral lymphocytes, PGE has been shown to increase cAMP concentrations by stimulating adenylate cyclase. This effect is not modified by β -adrenergic blocking agents. PGF_{2 α} has been shown to have less or no cAMP stimulating activity relative to PGE₂. In addition, PGF_{2 α} has been shown, in other tissues, to raise levels of cyclic GMP, a known antagonist of cAMP.¹¹⁴

There has also been indirect evidence implicating an effect of PG's on the calcium-dependent phase of smooth muscle contraction. PG's have been shown to alter the membrane permeability of calcium in smooth muscle preparations.¹¹⁶ The potency of PG's to affect smooth muscle's contractile state has been related to the calcium concentration in the surrounding medium.¹¹⁴ Carsten has provided evidence that PG's stimulate muscle contraction by increasing calcium release from smooth muscle sarcoplasmic reticulum.¹¹⁷

Recent evidence has suggested that PG's may play a role in bronchial asthma and EIB. Mathe et al demonstrated that aerosol PGF_{2 α} causes bronchoconstriction in both asthmatic and healthy subjects, but asthmatics hyperrespond.¹¹⁸ They suggested that bronchoconstriction in asthmatics may be due to local effects of PGF_{2 α} . Parker and Synder, however, have suggested that this may only be a response of irritant receptors to the aerosol propellant.¹¹⁹ Allegra et al showed that the baseline peripheral plasma PGE levels in asthmatics is significantly higher than in nonasthmatics.¹²⁰ They postulated that this is a compensatory mechanism in asthmatics to raise cAMP levels in bronchial smooth muscle cells (possibly to overcome lower baseline

cAMP levels due to decreased β -adrenergic responsiveness). Later work by this same laboratory, however, failed to demonstrate changes in peripheral PG levels after exercise in patients with EIB, but these measurements did not reflect local bronchial changes in PG concentrations.¹²¹ Indirect evidence against a role for PG's in asthma and EIB include the inability of sodium cromoglycate to prevent PGF_{2 α} - induced bronchoconstriction¹²² and the failure of indomethacin, an inhibitor of prostaglandin synthetase, to prevent EIB at a dose of two hundred milligrams daily.¹²³

VI. LITERATURE REVIEW: ASCORBIC ACID

A. Background

Lind demonstrated in 1757 that an essential dietary factor caused scurvy, a common disease of seamen of his day.¹²⁴ He showed that fresh vegetables and citrus fruits prevented this disorder. Holst and Frolich induced scurvy in guinea pigs, and this experimental model aided Szent Gyorgy, an ingenious Hungarian researcher, to isolate ascorbic acid and to determine that it is a single crystalline substance. Reisseissen, in the early nineteenth century, first described the symptoms of "convulsive asthma" in severely scorbutic patients,¹²⁵ suggesting that vitamin C might somehow prevent symptoms of asthma.

Ascorbic acid is synthesized from glucose by many species of plants and animals via the glucuronic acid pathway. The ability to synthesize this molecule is absent in invertebrates, insects, and fish. Amphibians gained the synthetic capacity in their kidneys, and this ability was transferred to the reptiles and the lower orders of birds. The site of vitamin C biosynthesis was transferred to the liver of mammals and the more evolved birds. Finally, the capacity to produce this molecule was lost by the guinea pig, the flying mammals, the monkey, man, and a number of highly evolved passeriformes birds.^{126,127} Some have suggested that the ability to synthesize this chemical was developed by amphibians as they entered terrestrial life because ascorbic acid functioned in some manner to overcome the more stressful conditions of life on land.¹²⁶ The single gene mutation postulated for the loss of this biosynthetic capacity might not have been lethal as ascorbic acid was present in the diets of the mutant animals.¹²⁶

H. The Relation of the Biochemical Effect of Vitamin C to Oxidation/Reduction Potential

Ascorbic acid (AA) is an α -keto-lactone with the chemical formula

$C_6H_6O_8$.¹²⁸ It is readily oxidized to dehydroascorbic acid (DHAA) via an intermediate compound, 3-monodehydroascorbate.^{126,129,130} This intermediate form has been shown to have free radical properties.¹³¹ Foerster et al demonstrated that mixing AA and DHAA at an acid pH leads to formation of this ascorbic free radical (AFR).¹³² Lagercrantz found AFR production in solutions of ascorbate and dissolved oxygen in the pH range of 6.6 to 9.6.¹³³ Therefore, its ability to remove single electrons from biologically active molecules and transform them into free radicals may allow AA to participate in many enzymatic reactions.¹³⁴

AA can easily be oxidized by cytochrome oxidase plus cytochrome c, and DHAA can be reduced by glutathione. Therefore, some feel that AA may serve to maintain sulphhydryl-containing enzymes reduced by its ability to take part in oxidation/reduction coupling.¹²⁹ Intake of large amounts of AA raises the tissue sulphhydryl concentration, suggesting that ascorbate aids in the reduction of disulfides.¹³⁵ In support of this theory, Price demonstrated that agents used to reduce sulphhydryls can be replaced with ascorbate.¹³⁶ Furthermore, the involvement of the AFR increases the ability of AA to reduce disulfide bonds as compared to simple oxidation/reduction coupling.¹³⁷

C. Ascorbic Acid and Anaphylaxis

Interest in a role for AA in the prevention of asthma developed from previous work suggesting an anti-anaphylactic effect of vitamin C. However, the results of these studies have ranged from the demonstration of marked protection to no effect at all, depending on the system studied and the dosage of vitamin C. Hoffman reported that AA had a protective effect against anaphylaxis in mice.¹³⁸ Herxheimer found no protection from

anaphylactic shock in guinea pigs treated with ten to twenty milligrams per kilogram of AA intramuscularly.¹³⁹ Dawson and West, however, demonstrated that AA (200 milligrams per kilogram) before antigen challenge protected guinea pigs from anaphylactic shock.²³ Guirgis confirmed this finding and showed that vitamin C (200 milligrams intramuscularly or intraperitoneally) extended the time from inhalation of histamine or antigen aerosol to onset of convulsions in guinea pigs.¹⁴⁰

D. Ascorbic Acid and Asthma

As early as 1938, Hunt suspected that AA might offer protection to patients with bronchospasm.²² He reasoned that adrenal medullary insufficiency may somehow be involved in this disease based on the following arguments. Vitamin C is found in significant quantities in the adrenal gland. In fact, Szent Gyorgy used the adrenal as a source when he first isolated AA. Hypersensitivity to proteins can be demonstrated in both adrenalectomized dogs and in asthmatics. Furthermore, eosinophilia is common both in asthmatics and patients with Addison's disease. Finally, adrenaline, well known for its ability to counteract bronchospasm, is produced in the adrenal medulla. However, he found that daily administration of AA (one hundred milligrams orally for up to eight weeks in asthmatic clinic patients did not improve the amount of their wheezing or the frequency of their attacks. In addition, during asthmatic episodes, 500 to 800 milligrams of vitamin C intramuscularly or intravenously did not decrease symptoms over a period of twenty to thirty minutes.²²

Stimulated by their earlier work with anaphylactic reactions in guinea pigs, Dawson and West studied AA's protective effect against

bronchospasm in these animals. They demonstrated that 500 milligrams per kilogram intravenously prevented bronchoconstriction induced by intravenous 5-hydroxytryptamine, bradykinin, or histamine. In addition, reducing the dose of AA led to a proportional increase in the amount of bronchospasm. β -adrenergic blockade did not affect AA's protective action.¹⁴

Zuskin, Lewis, and Bouhuys studied AA's effect both in histamine-induced airway constriction in healthy human subjects and in guinea pig tracheal strips in vitro.²⁸ Using PEFV curves, they measured the maximal flow rates at fifty and twenty-five percent of vital capacity as parameters of airway obstruction. They demonstrated that five hundred milligrams of AA orally, in contrast to placebo, reduced the amount of bronchospasm provoked by histamine aerosol. Propranolol did not reduce this protection against bronchospasm. Adding AA to an organ bath containing guinea pig tracheal muscle resulted in a dose-related relaxation. AA also reduced histamine-induced contractions in this in vitro system. Both of these effects of vitamin C in guinea pig tracheal muscle in vitro were prevented by β -blockade. They concluded that the differences in response to β -blockade are explained either by a species difference in the mechanism of action of vitamin C in guinea pigs vs man or that the dose of AA in man was sufficient to overcome the β -blockade, in contrast to the guinea pig model. However, when they repeated this experiment using MEF40% (P) to measure bronchospasm in mild asthmatics, no protective effect of AA could be demonstrated.²⁹ Kordansky et al, in an independent study, showed that vitamin C provided no improvement in ragweed antigen-induced bronchospasm.³⁰

Zuskin, Valic, and Bouhuys have provided more evidence for AA's ability to prevent bronchoconstriction by their research with textile dust

exposure. Valic and Zuskin demonstrated that prior administration of vitamin C (five hundred milligrams orally) attenuated the bronchospasm induced by flax dust exposure as measured by FEV₁ and Vmax 50%. This protective effect was also provided by pretreatment with metaproterenol sulfate (alupent^R) inhaler or diadril, an antihistamine.¹⁴² Zuskin and Bouhuys found that either AA (1 gram) or methdilazine-hydrochloride (8 milligrams), an antihistamine, reduced bronchoconstriction induced by hemp dust extract aerosol inhalation as measured by MEF40% (P). Furthermore, they demonstrated that propranolol potentiated the bronchospastic properties of this textile dust, suggesting that the balance of vagal and β-adrenergic activity is somehow involved.¹⁴³

E. Postulated Mechanisms of Action of AA's Influence on the Contractile State of Smooth Muscle and Its Potential Effect in Asthma

1. Direct Effect on Smooth Muscle

Some researchers theorize a direct effect of vitamin C upon smooth muscle. They provide both direct evidence for an effect of AA as a modulator of smooth muscle tone or indirect evidence, such as the ability of AA to suppress the effect of spasmogens. These arguments have been somewhat confusing as different agents have been shown to have varying effects, depending both on the type of smooth muscle (i.e. ileal, tracheal, etc.) or the species of animal studied. Therefore, one can only speculate from this evidence that AA may have a direct effect upon smooth muscle in the regulation of its contractile state.

a. Early Work

The response of guinea pig ileal muscle to spasmogens has been shown to vary at different times of the year.²⁵ Dawson et al suggested that variations in the AA content of the diet might account for

these contradictions. Their group has found that the ileal smooth muscle of guinea pigs fed with an AA supplemented diet was ten times more sensitive to acetylcholine and histamine-induced muscle contraction than the muscle of those animals fed a normal diet.²⁵ In a later experiment, they confirmed that high doses of AA led to inhibition of contraction by these spasmogens, while lower doses actually potentiated their effect.

They have also demonstrated that sodium ascorbate has a direct effect leading to contraction of guinea pig ileal muscle that is probably not related to its ability to alter oxidation/reduction potential since sodium metabisulphite and thiourea known reducing agents, were by themselves unable to induce contractions.²⁷ Furthermore, they confirmed their earlier *in vivo* experiments demonstrating a protective effect of AA against bronchospasm induced by histamine, bradykinin, 5-hydroxytryptamine, and acetylcholine in guinea pigs. Pretreatment with vitamin C was shown to inhibit bronchial smooth muscle contraction for fifteen minutes, followed by potentiation for fifteen minutes before return to control levels. These effects were unchanged by depleting the animal's tissues of catecholamines or 5-hydroxytryptamine or by β -blockade, suggesting a direct effect upon the tracheal smooth muscle.²⁷

b. Direct Effect of AA on Smooth Muscle - Oxidation/Reduction Potential

In contrast, Ghouri et al suggested that, in some animal models, AA modulates the response of smooth muscle to spasmogens through an alteration of oxidation/reduction potential.³¹ Using rabbit and guinea pig ileum, rabbit aortic strips, and rabbit atria, the authors showed that

reducing agents, such as AA and sodium bisulfite, attenuated the normal contractile effect of acetylcholine, norepinephrine, L-isoproterenol bitartrate, and 2-aminoethanol, whereas the oxidizing agent ammonium persulfate inhibited muscle relaxation by norepinephrine and magnesium. They further demonstrated that those compounds that produce muscle relaxation, including AA, were able to reduce the oxidized form of cytochrome c. Those agents that produced contraction did not show this effect. Therefore, they postulated that the aromatic ring portion of biogenic amines act as reducing agents and cause muscle relaxation, while the aminoethyl side chains, through their oxidizing potential, induce contraction. According to this scheme, AA's capacity to relax certain types of smooth muscle would be a function of its reducing capacity.

c. Direct Effect of AA on Smooth Muscle-Calcium Flux Theory

Joiner proposed that the direct effect of AA on smooth muscle might be due to an effect on cellular calcium flux.²⁶ He demonstrated that sodium ascorbate stimulated contraction in guinea pig ileal muscle in vitro, and this effect was partially mimicked by disodium ethylenediamine, a calcium chelating agent. Sodium ascorbate also potentiated acetylcholine-induced muscle contractions. Joiner suggested that the direct stimulation by AA may be due to interactions with calcium in the extracellular space which leads to decreased levels of calcium near the cell membrane. In response to this decreased local calcium concentration, calcium located within the cell membrane (and functioning to stabilize it) dissociates and moves extracellularly. This leads to a change in permeability and an influx of free calcium or

calcium-ascorbate from the extracellular pool. He further speculated than an ascorbate-calcium interaction in the cell membranes may play a role in the potentiation of acetylcholine-induced contractions by ascorbate.

Others have demonstrated an effect of AA on calcium flux in systems other than smooth muscle. Garcia-Sancho et al have demonstrated that extracellular ascorbate leads to increased potassium flux across the human red blood cell membrane, an effect similar to that produced by increased intracellular calcium (the Gardos effect).¹⁴⁴ This change in potassium flux was also produced by other electron donors, including reduced glutathione. These authors suggested that ascorbate might alter the oxidation/reduction state of some membrane component leading to a change in intracellular calcium which thereby elicits rapid potassium movements. From these various experiments implicating a calcium-ascorbate interaction in smooth muscle and other systems, one can only state that a direct effect upon smooth muscle elicited by AA is potentially mediated by an influence upon calcium flux, possibly by modulating the oxidation/reduction status of the membrane of the cell or its internal organelles.

2. AA and Histamine Metabolism

Interested in the protective effect of AA against anaphylaxis, Dawson and West studied the effect of vitamin C on histamine metabolism. They demonstrated that a scorbutic state caused increased histamine levels in guinea pigs as measured by levels in the urine and various tissues. Furthermore, this was shown to be unrelated to a change in histaminase activity.²³

A series of experiments by Chatterjee et al has helped to elucidate AA's influence on histamine metabolism. They confirmed that an

AA-free diet in guinea pigs leads to increased levels of histamine in blood, urine, and several other tissues. These increases could be returned to normal levels by a single dose of vitamin C (five milligrams per one hundred grams of body weight). They also demonstrated that there was no change in histamine formation, histaminase activity, or histamine release from mast cells, suggesting a direct effect of AA on histamine degradation.¹⁴⁵ They further reported that histamine levels could be raised in rats and guinea pigs by various stressful conditions, including vaccines, toxoids, cold, heat, pregnancy, drugs, and several diets. AA was able to decrease urinary histamine levels in these animals, indicating *in vivo* detoxification.^{146,147}

The chemical mechanism of this histamine breakdown induced by vitamin C was also addressed by Chatt erjee *et al.* Oxidation of AA to DHAA in the presence of histamine, and copper ion as a catalyst, led to the decomposition of histamine to aspartic acid, ammonia, and carbon dioxide.³² Monodehydroascorbic acid, the intermediate between AA and DHAA, appeared to be the reactive compound.^{32,148} This reaction was demonstrated in biological materials, such as homogenates or slices of different organs of the rat or guinea pig.³²

3. AA and Cyclic Nucleotides

Ascorbic acid has been shown to have an effect on the levels of the cyclic nucleotides cAMP and cGMP in various experimental models. However, confusion has arisen because the changes in cyclic nucleotide levels induced by vitamin C, and the postulated mechanisms involved, have varied in the different systems studied.

Goldberg *et al.* found that guanylate cyclase activity was stimulated by AA or DHAA in intact guinea pig splenic cells. This activation

appeared to require an oxidant effect. Because DHAA had this action in disrupted cells while AA did not, the authors postulated that AA was converted to DHAA or a free radical intermediate in intact cells and acquired the properties of an oxidizing agent.¹⁴⁹ This theory suggesting intracellular modification of vitamin C was supported by the finding that guanylate cyclase, as opposed to adenylate cyclase, was not predominantly found in the cell membrane, but rather was distributed in soluble and subcellular fractions.¹⁴⁹

Schoepflin et al studied AA and cyclic nucleotide levels in human platelets. They suggested that AA raises cGMP levels in platelets in a different manner than that by which cholinergic agents raise the level of this cyclic nucleotide in other systems, such as smooth muscle. This was based on the finding that AA's effect, unlike the cholinergic mechanism, was independent of calcium levels.^{150,151} They, too, suggest that oxidation might favor an increase in guanylate cyclase acitivity.¹⁵¹

Atkinson et al studied the effect of AA on cyclic nucleotide metabolism in human lymphocytes and also found increased levels of cGMP in cells exposed to vitamin C. They found no inhibition of cAMP or cGMP-phosphodiesterase (PDE) activity in this system. They considered several theoretical mechanisms for indirect activation of guanylate cyclase, including auto-oxidation of AA to produce hydrogen peroxide, liberation of fatty acids, prostaglandin production, modulation of redox potential, and free radical formation. However, definitive evidence could not be provided for any of these potential explanations.³⁵

Lewin has suggested a scheme for the involvement of vitamin C in cyclic nucleotide metabolism.¹⁵² He has postulated that AA might aid

in the production of epinephrine at three separate stages and therefore lead to increased activation of adenylate cyclase. These steps include the involvement of ascorbate as a cofactor in the conversion of dopamine to norepinephrine, the ability of the reducing power of AA to aid in methylation of norepinephrine to form epinephrine, and the protection offered by AA to maintain epinephrine in the active reduced state. In addition, he has suggested that AA can inhibit PDE's ability to hydrolyze cAMP by competitively binding to the enzyme. Both cAMP and AA possess ring structures with negatively charged oxygen molecules that might fit into the enzyme's active site. This inhibition of PDE has been demonstrated by other researchers.¹⁵³ Tisdale demonstrated that both AA and DHAA are reversible inhibitors of cAMP - PDE using enzyme obtained from Walker carcinoma cells in rats.¹⁵⁴ In conclusion, Lewin proposed that vitamin C may increase cAMP levels by both increasing available epinephrine to stimulate cAMP production and by inhibiting its enzymatic hydrolysis.

4. AA and Prostaglandins

Nugteren and Hazelhof demonstrated the presence of a compound (which they designated PGR₂) that is a common intermediate in the formation of both PGE₂ and PGF_{2α} in sheep vesicular glands. They also showed that the isomerization of this intermediate to form PGE₂ required glutathione.¹⁵⁵ Lands et al confirmed this finding and showed that addition of reduced glutathione favored production of PGE as opposed to PGF.¹⁵⁶

Stimulated by evidence that reduced glutathione might lead to the preferential formation of PGE₂ from cyclic endoperoxide, Puglisi et al studied the effect of AA on PG metabolism in guinea pig and human uterine myometrium. In this system, both PGE₂ and PGF_{2α} stimulate muscle contraction.

The authors found that AA increased smooth muscle contraction, and this was prevented by indomethicin and eicosatetraynoic acid, two inhibitors of PG synthesis. Therefore, they concluded that AA's stimulatory effect was due to PG synthesis. Furthermore, they demonstrated that AA led to a large increase in PGE₂ levels and suggested that vitamin C induced preferential synthesis of PGE₂.³⁴ Pugh et al observed an inhibitory effect of AA on PGF levels in guinea pig uterine homogenates³³ but did not measure changes in the yield of other PG's.

Puglisi et al studied vitamin C and PG's in guinea pig tracheal smooth muscle. They reported that AA antagonized the airway constriction produced by PGF_{2 α} . Furthermore, they demonstrated that AA induced direct smooth muscle relaxation in this system which can be blocked by inhibitors of PG synthesis. This relaxation was found to be proportional to PGE₂ levels measured in the muscle tissue. Muscle samples from animals fed with an AA-free diet contained smaller amounts of PGE₂ and larger quantities of PGF_{2 α} as compared to animals fed a normal diet. Therefore, these authors confirmed the finding that AA leads to enhanced synthesis of PGE₂ as opposed to PGF_{2 α} , and they reaffirmed the postulate that vitamin C's effect may be secondary to its ability to maintain glutathione in the reduced state.^{157, 158}

VII. DISCUSSION

A. Discussion of Results

1. Effect of AA

Our results demonstrate that AA provides protection against airway obstruction in patients with EIB. We found that AA partially reversed the decrement after exercise in all five parameters of pulmonary function and produced a statistically significant attenuation of the post-exertional fall in FVC and FEV₁.

FVC and FEV₁ tend to represent the state of the large airways as opposed to other parameters, such as MEF40% and MEF40% (P), which correlate with small airway status. One explanation for this discrepancy in the response to vitamin C is that the relief of post-exertional airway obstruction offered by AA is predominantly limited to the large airways. Alternatively, the effect of AA may have been more widespread with subtle improvements in smaller airways that were not detected by our parameters because of the large variability of flow rates at low lung volumes.

Previous work has demonstrated contradictory data concerning the ability of vitamin C to reverse histamine-induced airway obstruction as measured by flow rates at low lung volumes using PEFV curves.^{28,29} Our results agree with those of Kreisman *et al*, as no significant change in MEF40% and MEF40% (P) were detected after treatment with AA versus placebo. However, we have demonstrated that AA attenuates the post-exertional decrease in parameters of large airway function in patients with EIB. These parameters representing large airways were not addressed in these previous studies with

histamine challenge. In a separate study by Zuskin, AA was shown to partially prevent decreases in FEV₁ after exposure to flax dust, suggesting that the site of action for AA in this experimental model may be similar to that in EIB.

Kordansky et al found that AA did not protect against ragweed antigen-induced bronchospasm as measured by changes in FEV₁.³⁰ This may suggest that the mechanism for airway obstruction produced by antigen challenge is different from that produced by exercise, as AA was effective in attenuating decreases in FEV₁, after exercise. It is well known that mast cell degranulation is involved in antigen-induced asthma. If mast cells are also responsible for EIB, AA may be acting at a stage of mast cell function unrelated to its role in antigen-induced asthma.

2. Response to Exercise

Data from the first experimental day reveals that our subjects with EIB demonstrated bronchoconstriction of both large and small airways. However, review of the individual responses of asthmatic subjects shows agreement with the finding of Schachter et al⁵⁰ that patients with EIB separate into two distinct groups. Whereas all subjects had large decrements in small airway flow rates (MEF40%, MEF40% (P)), one group had only minimal decreases (< 10%) in parameters measuring large airway function (FVC, FEV₁, PEFR), while a second group demonstrated more severe obstruction in the large airways. We also confirmed the finding of Schachter et al⁵⁰ that MEF40% (P) is the most sensitive measure of post-exertional airway obstruction.

In agreement with previous studies, nonasthmatics were shown to have no significant change in pulmonary function after exercise.⁵⁰

This is in contrast to the ability to detect changes in airway dynamics using PEFV curves in healthy subjects with histamine and methacholine challenges.⁶⁴

Both groups revealed improvement in airway dynamics after metaproterenol as compared to baseline measurements ($p < .05$ for FEV_1 , PEFR, MEF40%, MEF40% (P) in asthmatics; $p < .05$ for MEF40% and MEF40% (P) in controls). This suggests that metaproterenol is functioning to dilate both large and small airways in the asthmatics, whereas its bronchodilating effect can only be detected in the small airways of healthy subjects.

B. Proposed Models

This demonstration of an antibronchospastic action of vitamin C, an agent able to modulate the effect of several proposed mediators of EIB and with no known influence on the ANS, strongly supports the mediator theory for the etiology of post-exertional asthma. However, the ANS might still play an auxillary role in regulating the responsiveness of the bronchial smooth muscle to mediators by altering baseline cAMP levels in smooth muscle cells and mast cells. Furthermore, AA's effect may not require a chemical mediator, as vitamin C itself has been shown to directly alter smooth muscle tone.

This protective effect of AA against EIB helps to direct our attention toward those suspected mediators that have been shown to be modulated by vitamin C. Although we have not rigorously eliminated other theories, we have provided sufficient evidence with our review of the literature to narrow the field and postulate certain possible mechanisms. Figure 21 displays four theories to explain the antibronchospastic properties

of AA in patients with EIB.

a. AA as an Antihistamine

Histamine has long been known to stimulate bronchial smooth muscle contraction. There is much evidence to support the theory that the antihistaminic property of vitamin C is central to its role in attenuating EIB. Histamine levels have been demonstrated to be increased in the arterial blood of patients with EIB, and antihistamines have been shown to reduce their symptoms. Chatterjee et al have demonstrated the ability of AA to detoxify histamine both *in vitro* and *in vivo*.

b. Direct Effect of AA on Smooth Muscle Tone

A direct effect of vitamin C on smooth muscle cells to relieve bronchospasm can also be postulated. Evidence for this theory is both sparse and indirect. Furthermore, work in this area has been confused by different responses found in different types of smooth muscle. Nevertheless, AA has been shown to both change the response of smooth muscle to spasmogens and to directly alter smooth muscle tone. It has been postulated that these effects are mediated by changes in calcium flux across the smooth muscle cell membrane or by an alteration of the oxidation-reduction potential at the site of action of neurotransmitters. With this indirect, and at times contradictory, evidence, one might speculate that AA alters smooth muscle tone in EIB directly by one or both of these postulated mechanisms.

c. Cyclic Nucleotides

As discussed previously, increases in cAMP levels have been associated with bronchodilatation, suggesting that this cyclic nucleotide might function to prevent bronchospasm. Furthermore, PDE

inhibitors have been shown to relieve airway obstruction in asthmatics. Although no direct effect has been demonstrated, it is postulated that cAMP in some way prevents smooth muscle contraction.

Lewis' theory for vitamin C's ability to modulate cyclic nucleotide levels emphasizes the rise in cAMP levels induced by AA and ignores the confusing data with cGMP and guanylate cyclase. Both by maintaining high levels of epinephrine (which stimulates adenylyl cyclase) and by inhibiting PDE, he theorizes that AA might raise cAMP levels in smooth muscle cells and prevent contraction. However, this evidence does not strictly support only the proposition that cyclic nucleotide levels in smooth muscle cells regulate airway tone in EIB. By raising cAMP levels, AA also alters the potential for mast cell degranulation and release of several potential mediators, such as histamine, SRS-A, and kinins. Therefore, vitamin C's ability to maintain increased levels of cAMP in smooth muscle cells may only be an associated finding, while its real effect of decreasing the symptoms of EIB might be due to prevention of mast cell degranulation.

d. PG's

PG's have been shown to modulate airway tone in animals and in man. PGE_2 is a bronchodilator, while $\text{PGF}_{2\alpha}$ stimulates bronchoconstriction.

Because AA leads to the preferential production of PGE_2 as opposed to $\text{PGF}_{2\alpha}$, it has been suggested that regulation of the ratio of these PG's is central to AA's ability to reduce EIB. This action of vitamin C might be mediated by activation of adenylyl cyclase or by alteration of calcium flux across smooth muscle cell membranes or the membranes of the

sarcoplasmic reticulum.

C. Speculation and Further Research

This *in vivo* study has demonstrated the ability of AA to decrease airway obstruction precipitated by exercise. As is usually the case in clinical research, theories of biochemical mechanisms are difficult to rigorously defend based on our results, and one easily feels that he or she is working with a "black box." However, by reviewing the literature of both the *in vivo* and the *in vitro* biochemical effects of AA in the prevention of bronchospasm, we have been able to focus attention on the four theories discussed above.

The proposal that AA decreases EIB by degrading histamine seems sound, as histamine has been closely linked to the production of bronchospasm in other forms of asthma. In order to test this hypothesis, measurements of plasma histamine levels might prove useful. One possible experiment could involve exercising patients with EIB after AA or placebo, and attempting to correlate changes in PFT's induced by vitamin C with alterations in plasma histamine levels.

The final common event in all of these theories is contraction of smooth muscle. Because calcium fluxes have been shown to be involved in all forms of muscle contraction, the suggestion that changes in calcium permeability are involved in the attenuation of EIB by vitamin C seems reasonable. This may be a direct action of AA or may be mediated by PG's or cyclic nucleotides. Some researchers have suggested that changes in cAMP and cGMP levels might only be associated with variations in smooth muscle tone and may not actually be causative.¹⁵⁹

Future biochemical investigation should be directed at the mechanisms of modulation of calcium movements in an attempt to discern the relative involvement of cyclic nucleotides, PG's, or AA itself.

TABLE 1

<u>Subject</u>	<u>Age (years)</u>	<u>Ht (cm)</u>	<u>Wt (kg)</u>	<u>Anthropometric Data:</u>			<u>Asthmatics</u>	<u>Dyspnea*</u> (0 → 4)
				<u>Sex</u>	<u>Allergies</u>	<u>Smoking</u>		
1	30	165	77	F	+	-	-	2-3
2	24	183	63	F	+	-	-	3
3	33	180	77	M	+	-	-	1
4	14	165	51	M	+	+	-	1
5	25	180	61	M	+	-	-	1-2
6	26	163	87	F	+	-	-	0
7	21	163	46	F	+	+	-	3
8	31	166	55	F	+	-	-	3
9	24	170	61	F	+	-	-	3
10	27	192	90	M	+	-	-	1
11	25	165	64	F	+	-	-	2
12	27	193	91	M	+	-	-	1
\bar{x}	26	173	66				1.8	
σ_{n-1}	5	10	15				1.1	

* Dyspnea Scale:

- 0 - 30-40 minutes of strenuous exercise before dyspneic (i.e., jogging)
- 1 - 10-15 minutes of strenuous exercise before dyspneic (i.e., jogging)
- 2 - 10-15 minutes of moderate exercise before dyspneic (i.e., volleyball)
- 3 - 10-15 minutes of minimal exercise before dyspneic (i.e., walking)
- 4 - dyspneic at rest

PFT's Observed & Expected: Asthmatics

(Baseline Without Drugs)

<u>Subject</u>	FVC (liter)	FVC Expected	% of Expected (liter)	FEV _{1.0} (liter)	FEV _{1.0} Expected	% of Expected (liter/Sec)	PEFR (liter/Sec)
1	3.9	3.9	100.0	3.2	3.1	103.2	7.3
2	5.0	4.8	104.2	2.9	3.9	74.4	5.7
3	4.2	5.4	77.8	1.9	4.2	45.2	4.8
4	3.4	4.0	85.0	2.2	3.3	66.7	4.8
5	5.4	5.6	96.4	3.1	4.4	70.5	6.6
6	3.7	3.9	94.9	2.9	3.1	93.5	6.1
7	3.5	4.0	87.5	3.2	3.3	97.0	6.1
8	2.8	3.9	71.8	1.6	3.1	51.6	3.4
9	3.6	4.2	85.7	2.9	3.4	85.3	6.2
10	5.7	6.2	91.9	4.0	4.8	83.3	9.6
11	3.4	4.0	85.0	2.3	3.2	71.9	5.5
12	3.7	5.7	64.9	2.6	4.5	58.3	5.5
\bar{x}				87.1		75.1	
$\sigma_{\bar{n}-1}$				11.5		18.1	

PFT's Observed & Expected: Asthmatics (Cont.)

(Baseline Without Drugs)

<u>Subject</u>	<u>PEFR Expected (liter/Sec)</u>	<u>% of Expected</u>	<u>MEF40% (liter/Sec)</u>	<u>MEF40% (P) (liter/Sec)</u>	<u>Vmax50% (liter/Sec)</u>	<u>Vmax50% Expected (liter/Sec)</u>	<u>% of Expected</u>
1	6.5	112.3	3.1	2.7	4.1	3.8	109.0
2	7.3	78.1	1.5	1.3	2.0	4.3	46.5
3	9.6	50.0	1.0	0.7	1.3	4.9	26.5
4	7.2	66.7	1.3	1.2	1.7	4.3	39.5
5	9.8	67.3	1.8	1.4	2.2	5.2	42.3
6	6.5	93.8	2.8	1.9	3.5	3.9	89.7
7	6.6	92.4	4.2	4.0	4.9	4.9	122.5
8	6.5	52.3	0.8	0.5	1.1	3.8	28.9
9	6.8	91.2	2.0	2.0	2.9	4.0	72.5
10	10.5	91.4	2.8	2.3	3.7	5.4	68.5
11	6.6	83.3	1.8	1.8	2.1	4.0	52.5
12	10.0	55.3	1.9	2.8	2.4	5.2	<u>46.6</u>
			77.8				62.1
			19.6				31.1
			\bar{x}				$\sigma_{\bar{y}-1}$

TABLE 3

<u>Subject</u>	<u>Anthropometric Data:</u>	<u>Healthy Subjects</u>			<u>Dyspnea (0 → 4)</u>
	<u>Age (year)</u>	<u>Ht(cm)</u>	<u>Wt(kg)</u>	<u>Sex</u>	
A	24	180	68	M	-
B	20	173	73	M	-
C	23	173	58	F	-
D	25	176	68	M	-
E	23	188	67	M	+
F	23	178	74	M	-
G	22	160	50	F	-
H	36	170	75	M	-
I	35	183	74	M	-
J	21	176	73	M	-
X	24	176	68		0.1
$\sigma n-1$		4	8		0.3

PFT's Observed & Expected: Healthy Subjects

(Baseline Without Drugs)

<u>Subject</u>	FVC (liter)	FVC Expected	% of Expected (liter)	FEV _{1.0} (liter)	FEV _{1.0} Expected	% of Expected (liter/Sec)	PEFR (liter/Sec)
A	4.5	5.6	80.4	3.5	4.5	77.8	8.9
B	4.0	5.3	75.5	3.3	4.3	76.7	7.3
C	4.0	4.4	90.9	3.9	3.6	108.3	9.1
D	3.7	5.3	69.8	3.4	4.3	79.1	10.5
E	4.6	6.1	75.4	4.4	4.8	91.7	8.4
F	5.2	5.5	94.5	4.9	4.4	111.4	10.0
G	3.7	3.8	97.4	3.2	3.1	103.2	7.9
H	4.9	4.7	104.3	4.5	3.7	121.6	11.4
I	4.8	5.8	82.8	4.5	4.5	100.0	6.2
J	3.9	5.4	<u>72.2</u>	3.2	4.4	<u>72.7</u>	7.1
—	X			84.3		94.3	
				11.8		17.1	

PFT's Observed & Expected: Healthy Subjects (Cont.)

Subject	PEFR Expected (liter/Sec)	(Baseline Without Drugs)			Vmax50% (liter/Sec)	% of Expected
		% of Expected	MEF40% (liter/Sec)	MEF40% (P) (liter/Sec)		
A	9.9	89.8	3.9	4.6	4.5	5.2
B	9.6	76.0	2.8	2.7	3.8	5.2
C	6.9	131.9	5.1	5.5	6.7	4.2
D	9.6	109.4	5.4	5.7	7.7	5.1
E	10.3	81.6	5.7	5.1	6.0	5.4
F	9.8	102.0	6.4	6.2	7.1	5.2
G	6.5	121.5	3.6	3.8	4.8	3.9
H	9.0	126.7	6.1	7.2	7.5	4.5
I	10.0	62.0	3.8	4.1	4.6	5.2
J	9.7	73.2	3.0	2.9	3.7	5.2

1167

36.0

σn=1

TABLE 5

Pulmonary Function Response to Exercise Without Drugs: Asthmatics

FVC (liter)

Subject	Before Exercise (Baseline)	% Baseline	Post-Exercise	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	3.9	100.0	3.9	100.0	3.8	97.4	4.0	102.6	
2	5.0	100.0	5.1	102.0	4.4	112.0	5.3	106.0	
3	4.8	100.0	5.3	110.4	4.3	89.6	5.7	118.8	
4	3.4	100.0	3.0	88.2	2.6	76.5	3.4	100.0	
5	5.4	100.0	5.3	98.1	4.7	87.0	5.5	101.9	
6	3.7	100.0	3.4	91.9	3.5	94.6	3.7	100.0	
7	3.5	100.0	3.5	100.0	3.2	91.4	3.5	100.0	
8	2.9	100.0	3.1	110.7	2.6	92.9	3.6	128.6	
9	3.6	100.0	3.8	105.5	3.4	94.5	3.8	105.5	
10	6.4	100.0	6.1	95.3	6.2	96.9	6.3	98.4	
11	3.1	100.0	3.2	103.2	2.6	83.9	3.1	100.0	
12	<u>3.7</u>	<u>100.0</u>	<u>3.8</u>	<u>102.7</u>	<u>3.1</u>	<u>83.8</u>	<u>3.3</u>	<u>89.2</u>	
\bar{x}	4.1	100.0	4.1	100.7	3.7	91.7	4.3	104.3	
σ_{n-1}	1.1	0.0	1.0	6.7	1.1	8.9	1.1	10.2	
				t=0.7		t=3.1			p < .01

TABLE 6

Pulmonary Function Response to Exercise Without Drugs: Asthmatics

Subject	FEV _{1.0} (liter)				Post Alupent	% Baseline
	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline		
1	3.2	100.0	3.2	100.0	2.9	90.6
2	2.9	100.0	3.1	106.9	2.5	86.2
3	2.4	100.0	2.7	112.5	2.0	83.3
4	2.2	100.0	1.9	86.4	1.5	68.2
5	3.1	100.0	3.5	112.9	2.5	80.6
6	2.9	100.0	2.9	100.0	2.8	96.6
7	3.2	100.0	3.3	103.1	3.1	96.9
8	1.6	100.0	1.8	112.5	1.4	87.5
9	2.9	100.0	2.9	100.0	2.3	79.3
10	4.8	100.0	4.7	97.9	4.5	93.7
11	1.9	100.0	2.0	105.3	1.4	73.7
12	2.6	100.0	2.4	92.3	2.0	76.9
\bar{x}	2.8	100.0	2.9	102.5	2.4	84.5
$\sigma n-1$	0.8	0.0	0.8	8.2	0.9	9.1
					t=1.1	0.9
					t=6.0	3.0
					p<.001	14.1
					t=2.3	109.3
					p<.05	

TABLE 7

Pulmonary Function Response To Exercise Without Drugs:
Asthmatic

<u>Subject</u>	<u>Before Exercise (Baseline)</u>	<u>% Baseline</u>	<u>PEFR (liter/Sec)</u>	<u>Immediate Post-Exercise</u>	<u>% Baseline</u>	<u>5 Minute Post-Exercise</u>	<u>% Baseline</u>	<u>Post Alupent</u>	<u>% Baseline</u>
1	7.3	100.0	7.8	106.8	6.9	94.5	8.2	112.3	
2	5.7	100.0	6.0	105.3	5.0	87.7	6.2	108.8	
3	5.4	100.0	5.9	109.3	4.2	77.8	5.8	107.4	
4	4.5	100.0	4.0	88.9	3.0	66.7	5.6	124.4	
5	6.6	100.0	8.1	122.7	5.8	87.9	8.4	127.3	
6	6.1	100.0	6.2	101.6	5.8	95.1	6.2	101.6	
7	6.1	100.0	6.4	104.9	5.9	96.7	6.6	108.2	
8	3.4	100.0	3.8	111.8	3.2	94.1	4.6	135.3	
9	6.2	100.0	6.4	103.2	5.3	85.5	6.7	108.1	
10	10.6	100.0	10.3	97.2	9.8	92.5	10.4	98.1	
11	4.1	100.0	4.4	107.3	2.9	70.7	4.9	119.5	
12	<u>5.5</u>	<u>100.0</u>	<u>5.1</u>	<u>92.7</u>	<u>4.2</u>	<u>76.4</u>	<u>4.4</u>	<u>80.0</u>	
X	6.0	100.0	6.2	104.3	5.2	85.5	6.5	110.9	
mean	1.8	0.0	1.9	8.9	1.9	10.2	1.8	14.6	
				t=1.7			t=5.0	t=2.6	
							p < .001	p < .05	

TABLE 8

Pulmonary Function Response to Exercise Without Drugs: Asthmatics

		MEF40% (liter/Sec)						
Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	3.1	100.0	3.2	103.1	2.4	77.4	3.8	102.6
2	1.5	100.0	1.7	113.3	1.0	66.7	2.0	133.3
3	1.4	100.0	1.7	121.4	0.7	50.0	1.9	135.7
4	1.4	100.0	1.1	78.6	0.5	35.7	1.6	114.3
5	1.8	100.0	2.6	144.4	1.0	55.6	2.8	155.6
6	2.8	100.0	2.8	100.0	2.3	82.1	3.2	114.3
7	4.2	100.0	4.6	109.5	2.9	69.0	4.6	109.5
8	0.8	100.0	1.2	150.0	0.6	75.0	1.9	237.5
9	2.0	100.0	2.2	110.0	1.4	70.0	3.4	170.0
10	3.6	100.0	3.5	97.2	2.9	80.6	4.1	113.9
11	1.0	100.0	1.2	120.0	0.5	50.0	1.4	140.0
12	1.9	100.0	1.6	84.2	0.8	42.1	1.1	57.9
	\bar{x}	100.0	2.3	111.0	1.4	62.9	2.7	133.7
σ_{n-1}	1.1	0.0	1.1	21.3	0.9	15.7	1.2	42.9
				t=1.8		t=8.3		t=2.7
						p < .001		p<.01

TABLE 9

Pulmonary Function Response to Exercise Without Drugs: Asthmatics

Subject	Before Exercise (Baseline)	% Baseline	MEF40% (P) (liter/Sec)	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	2.7	100.0	3.0	111.1	1.4	51.9	4.2	155.6	
2	1.3	100.0	1.6	123.1	0.8	61.5	2.4	184.6	
3	1.4	100.0	1.4	100.0	0.5	35.7	2.1	150.0	
4	1.4	100.0	0.9	64.3	0.5	35.7	1.6	114.3	
5	1.4	100.0	2.7	192.9	0.7	50.0	3.0	214.3	
6	1.9	100.0	2.1	110.5	1.4	73.7	3.2	168.4	
7	4.0	100.0	4.5	112.5	2.4	60.0	4.3	107.5	
8	0.5	100.0	1.1	220.0	0.4	80.0	2.4	480.0	
9	2.0	100.0	2.3	115.0	1.0	50.0	3.4	170.0	
10	3.6	100.0	3.3	91.7	2.5	69.4	4.5	125.0	
11	0.7	100.0	0.8	114.3	0.3	42.9	1.0	142.9	
12	2.8	100.0	1.8	64.3	0.9	32.1	1.4	50.0	
\bar{x}	2.0	100.0	2.1	118.3	1.1	53.6	2.8	171.9	
$n-1$	1.1	0.0	1.1	45.7	0.7	15.7	1.2	105.7	
						P < .001			p < .05
						t=10.5			t=2.4

TABLE 10

Pulmonary Function Response to Exercise Without Drugs: Healthy Subjects

		FVC (liter)			Post-Exercise			Post-Exercise			Post-Exercise		
Subject	Before Exercise (Baseline)	% Baseline	Post-Exercise	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post-Exercise	% Baseline
1	4.5	100.0	4.5	100.0	4.6	102.2	4.6	102.2	4.6	102.2	4.6	102.2	4.6
2	4.0	100.0	3.9	97.5	4.1	102.5	4.3	102.5	4.3	105.0	4.3	105.0	4.3
3	4.0	100.0	3.9	97.5	3.9	97.5	4.0	97.5	4.0	100.0	4.0	100.0	4.0
4	3.7	100.0	4.0	108.1	3.8	102.7	3.8	102.7	3.8	102.7	3.8	102.7	3.8
5	4.6	100.0	4.6	100.0	4.4	95.7	4.4	95.7	4.4	95.7	4.4	95.7	4.4
6	5.2	100.0	5.0	96.2	5.0	96.2	5.3	96.2	5.3	101.9	5.3	101.9	5.3
7	3.7	100.0	3.6	97.3	3.7	100.0	3.7	100.0	3.7	100.0	3.7	100.0	3.7
8	4.9	100.0	4.9	100.0	4.9	100.0	5.0	100.0	5.0	98.0	5.0	98.0	5.0
9	4.8	100.0	4.6	95.8	4.5	93.7	4.6	93.7	4.6	95.8	4.6	95.8	4.6
10	3.9	100.0	4.4	112.8	4.2	107.7	4.0	107.7	4.0	102.6	4.0	102.6	4.0
—													
X	4.3	100.0	4.3	100.5	4.3	99.8	4.4	99.8	4.4	100.4	4.4	100.4	4.4
$\sigma n-1$	0.5	0.0	0.5	5.6	0.4	4.2	0.5	4.2	0.5	3.1	0.5	3.1	0.5
						t=0.3				t=0.2			t=0.4

TABLE 11

Pulmonary Function Response to Exercise Without Drugs: Healthy Subjects

Subject	FEV _{1.0} (liter)						Post Alupent % Baseline
	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	
1	3.5	100.0	3.6	102.9	3.7	94.7	3.8
2	3.3	100.0	3.3	100.0	3.4	103.0	3.6
3	3.9	100.0	3.8	97.4	3.8	97.4	3.8
4	3.4	100.0	3.7	108.8	3.6	105.9	3.6
5	4.4	100.0	4.3	97.7	4.3	97.7	4.2
6	4.9	100.0	4.9	100.0	4.7	95.9	5.2
7	3.2	100.0	3.2	100.0	3.3	103.1	3.3
8	4.5	100.0	4.7	104.4	4.7	104.4	4.8
9	4.5	100.0	4.0	88.9	3.9	86.7	4.1
10	3.9	100.0	3.8	118.6	3.6	112.5	3.6
\bar{x}	3.9	100.0	3.9	101.9	3.9	100.1	4.0
σ_{n-1}	0.6	0.0	0.6	7.8	0.5	7.2	0.6
						t=0.0	t=1.7

TABLE 12

Pulmonary Function Response to Exercise Without Drugs: Healthy Subjects

Subject	PEFR (liter/Sec)						Post Alupent	% Baseline
	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline		
1	8.9	100.0	9.3	104.5	9.6	107.9	9.7	109.0
2	7.3	100.0	7.5	102.7	7.5	102.7	8.3	113.7
3	9.1	100.0	8.4	92.3	8.5	93.4	8.2	90.1
4	10.5	100.0	10.9	103.8	10.6	100.9	10.0	95.2
5	8.4	100.0	8.8	104.8	8.6	102.4	8.7	103.6
6	10.0	100.0	9.9	99.0	9.7	97.0	9.9	99.0
7	7.9	100.0	8.1	102.5	8.2	103.8	8.1	102.5
8	11.4	100.0	11.6	101.8	11.7	102.6	12.3	107.9
9	6.2	100.0	6.1	98.4	6.0	96.8	6.2	100.0
10	7.1	100.0	9.0	126.8	8.4	118.3	8.5	119.7
\bar{x}	8.7	100.0	9.0	103.7	8.9	102.6	9.0	104.1
$\sigma n-1$	1.6	0.0	1.6	9.0	1.6	6.9	1.6	8.8
				$t=1.3$			$t=1.2$	
							$t=1.5$	

Pulmonary Function Response to Exercise Without Drugs: Healthy Subjects

TABLE 13

		MEF40% (liter/Sec)			Post-Exercise			5 Minute Post-Exercise			Post Alupent			% Baseline		
Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	Post-Exercise	% Baseline	Post-Exercise	% Baseline	Post-Exercise	% Baseline	Post-Exercise	% Baseline	Post-Exercise	% Baseline	Post Alupent	% Baseline
1	3.9	100.0	3.9	100.0	4.2	107.7	4.7	120.5								
2	2.8	100.0	2.8	100.0	3.0	107.1	3.8	135.7								
3	5.1	100.0	5.8	113.7	5.6	109.8	6.0	117.6								
4	5.4	100.0	7.3	135.2	6.9	127.8	6.2	114.8								
5	5.7	100.0	5.9	103.5	5.5	96.5	5.6	98.2								
6	6.4	100.0	6.7	104.7	6.7	104.7	6.9	107.8								
7	3.6	100.0	3.6	100.0	3.8	105.6	4.1	113.9								
8	6.1	100.0	6.6	108.2	6.6	108.2	7.0	114.8								
9	3.8	100.0	3.8	100.0	3.7	97.4	4.1	107.9								
10	3.0	100.0	4.7	156.7	4.3	143.3	4.5	150.0								
	\bar{x}	4.6	100.0	5.1	112.2	5.0	110.8	5.3	118.1							
$\sigma n - 1$		1.3	0.0	1.5	19.0	1.4	14.2	1.2	14.8							
															t=2.4	t=3.9
															p < .05	p < .01

TABLE 14

Pulmonary Function Response to Exercise Without Drugs: Healthy Subjects

<u>Subject</u>	<u>Before Exercise (Baseline)</u>	<u>% Baseline</u>	<u>Immediate Post-Exercise</u>	<u>% Baseline</u>	<u>5 Minute Post-Exercise</u>	<u>% Baseline</u>	<u>Post- Alupent</u>	<u>% Baseline</u>
1	4.6	100.0	4.3	93.5	4.6	100.0	5.0	108.7
2	2.7	100.0	2.8	103.7	2.6	96.3	4.1	151.9
3	5.5	100.0	4.7	85.5	4.9	89.1	6.5	118.2
4	5.7	100.0	7.3	128.1	5.5	96.5	7.3	128.1
5	5.1	100.0	4.8	94.1	5.2	102.0	6.3	123.5
6	6.2	100.0	5.8	93.5	6.7	108.1	6.5	104.8
7	3.8	100.0	3.6	94.7	3.7	97.4	4.3	113.2
8	7.2	100.0	7.6	105.6	7.8	108.3	7.9	109.7
9	4.1	100.0	3.5	85.4	3.8	92.7	4.7	114.6
10	<u>2.9</u>	<u>100.0</u>	<u>4.4</u>	<u>178.9</u>	<u>3.9</u>	<u>149.3</u>	<u>3.6</u>	<u>124.1</u>
\bar{x}	4.8	100.0	4.9	106.3	4.9	104.0	5.6	119.7
σ_{n-1}	1.4	0.0	1.6	28.4	1.5	17.0	1.5	13.6
				t=0.7		t=0.8		t=4.6
							p<.01	

TABLE 15

Pulmonary Function Response to Placebo Vs. Drug: FEV_{1.0}, PEFR

<u>Subject</u>	<u>Before Exercise</u>			
	<u>FVC</u> <u>Placebo</u> <u>(liter)</u>	<u>FVC</u> <u>No Drug</u> <u>(liter)</u>	<u>FEV_{1.0}</u> <u>Placebo</u> <u>(liter)</u>	<u>FEV_{1.0}</u> <u>No Drug</u> <u>(liter)</u>
1	3.5	3.9	2.8	3.2
2	5.0	5.0	2.8	2.9
3	4.5	4.8	2.2	2.4
4	3.4	3.4	2.4	2.2
5	5.1	5.4	2.9	3.1
6	3.5	3.7	2.8	2.9
7	3.1	3.5	2.9	3.2
8	3.5	2.8	2.1	1.6
9	3.4	3.6	2.7	2.9
10	5.8	6.4	4.2	4.8
11	3.2	3.1	2.7	1.9
12	3.8	3.7	2.5	2.6
\bar{x}	4.0	4.1	2.8	2.8
σ_{n-1}	0.9	1.1	0.5	0.8
				1.2
				6.0
				6.0
				1.8
				5.5

TABLE 16

Pulmonary Function Response to Placebo Vs. No Drug: MEF40%, MEF40% (P), Vmax50%

<u>Subject</u>	<u>Before Exercise</u>			<u>Vmax50% Placebo (liter/Sec)</u>	<u>Vmax50% No Drug (liter/Sec)</u>	<u>MEF40% Placebo (liter/Sec)</u>	<u>MEF40% No Drug (liter/Sec)</u>	<u>Vmax50% Placebo (liter/Sec)</u>	<u>No Drug (liter/Sec)</u>
	<u>MEF40% Placebo (liter/Sec)</u>	<u>MEF40% No Drug (liter/Sec)</u>	<u>MEF40% (P) Placebo (liter/Sec)</u>						
1	2.5	3.1	2.1		2.7		3.4		4.1
2	1.4	1.5	1.3		1.3		1.9		2.0
3	1.1	1.4	1.0		1.4		1.4		1.8
4	1.6	1.4	1.7		1.4		1.8		1.7
5	1.8	1.8	1.8		1.4		2.2		2.2
6	2.8	2.8	2.6		1.9		3.6		3.5
7	3.7	4.2	4.1		4.0		4.6		4.9
8	1.1	0.8	1.0		0.5		1.5		1.1
9	2.1	2.0	1.9		2.0		2.7		2.9
10	2.8	3.6	2.2		3.6		3.9		4.4
11	2.8	1.0	3.0		0.7		3.6		1.5
12	<u>1.6</u>	<u>1.9</u>	<u>2.0</u>		<u>2.8</u>		<u>2.1</u>		<u>2.4</u>
\bar{x}	2.1	2.1	2.1		2.0		2.7		2.7
$\sigma n-1$	0.8	1.1	0.9		1.1		1.1		1.2

TABLE 17

Pulmonary Function Response to Placebo Vs. No Drug: FVC, FEV_{1.0}, PEFR

Subject	Immediate Post-Exercise											
	FVC Placebo (liter)	Δ FVC* Placebo ($\Delta\%$)	FVC No Drug (liter)	Δ FVC No Drug ($\Delta\%$)	FVC _{1.0} Placebo (liter)	Δ FEV _{1.0} Placebo ($\Delta\%$)	FEV _{1.0} No Drug (liter)	Δ FEV _{1.0} No Drug ($\Delta\%$)	PEFR Placebo (liter/sec)	Δ PEFR Placebo ($\Delta\%$)	PEFR No Drug (liter/sec)	Δ PEFR No Drug ($\Delta\%$)
1	3.7	+5.7	3.9	0.0	2.9	+3.6	3.2	0.0	7.2	+14.3	7.8	+6.8
2	4.8	-4.0	5.1	+2.0	2.8	0.0	3.1	+6.9	5.3	-3.6	6.0	+5.3
3	4.3	-4.4	5.3	+10.4	2.1	-4.5	2.7	+12.5	4.7	-4.1	5.9	+9.3
4	3.1	-8.8	3.0	-11.8	2.1	-12.5	1.9	-13.6	4.2	-19.2	4.0	-11.1
5	5.2	+2.0	5.3	-1.9	3.5	+20.7	3.5	+12.9	7.9	+25.4	8.1	+22.7
6	3.5	0.0	3.4	-8.1	3.0	+7.1	2.9	0.0	6.2	+5.1	6.2	+1.6
7	3.1	0.0	3.5	0.0	3.1	+6.9	3.3	+3.1	6.3	+5.0	6.4	+4.9
8	3.4	+2.9	3.1	+10.7	2.2	+4.8	1.8	+12.5	4.7	+9.3	3.8	+11.8
9	3.5	+2.9	3.8	+5.5	3.0	+11.1	2.9	0.0	6.6	+8.2	6.4	+3.2
10	6.0	+3.4	6.1	-4.7	4.6	+9.5	4.7	-2.1	9.9	+8.8	10.3	-2.8
11	3.2	0.0	3.2	+3.2	2.4	-11.1	2.0	+5.3	5.2	-20.0	4.4	+7.3
12	3.7	-2.6	3.8	+2.7	2.3	-8.0	2.4	-7.7	4.8	-15.8	5.1	-7.3
\bar{x}	4.0	-0.2	4.1	+0.7	2.8	+2.3	2.9	+2.5	6.1	+1.1	6.2	+4.3
σ_{n-1}	0.9	4.1	1.0	6.7	0.7	9.9	0.8	8.2	1.6	14.0	1.9	3.9

*Explanation of " Δ PFT" Notation: Δ FVC (Immediately Post-Exercise) = the change in FVC (as a percent of FVC Before Exercise) from Before Exercise to Immediate Post-Exercise

TABLE 18

Pulmonary Function Response to Placebo Vs. No Drug: MEF40%, MEF40% (P)

Subject	MEF40% Placebo (liter/Sec.)	Immediate Post-Exercise				ΔMEF40% Placebo (Δ%)	ΔMEF40% No Drug (Δ%)	ΔMEF40% Placebo (liter/Sec.)	ΔMEF40% No Drug (liter/Sec.)	ΔMEF40% No Drug (Δ%)
		ΔMEF40% Placebo (Δ%)	MEF40% No Drug (liter/Sec.)	ΔMEF40% No Drug (Δ%)	MEF40% Placebo (liter/Sec.)					
1	2.9	+16.0	3.2	+3.1	2.5	419.0	3.0			+11.1
2	1.4	0.0	1.7	+13.3	1.2	-7.7	1.6			+23.1
3	1.0	-9.1	1.7	+21.4	0.7	-30.0	1.4			0.0
4	1.1	-31.3	1.1	-21.4	1.0	-41.2	1.9			-35.7
5	2.4	+33.3	2.6	+44.4	2.9	+61.1	2.7			+92.9
6	3.3	+17.9	2.8	0.0	3.0	+15.4	2.1			+10.5
7	5.1	+37.8	4.6	+9.5	4.8	+17.1	4.5			+12.5
8	1.3	+18.2	1.2	+50.0	1.3	+30.0	1.1			+120.0
9	2.9	+38.1	2.2	+10.0	2.9	+52.6	2.3			+15.0
10	4.0	+42.9	3.5	-2.8	3.9	+43.6	3.3			-8.3
11	2.2	-21.4	1.2	+20.0	2.5	-16.7	0.8			+14.3
12	1.3	-18.8	1.6	-15.8	1.3	-35.0	1.8			-35.7
\bar{x}	2.4	+10.3	2.3	+11.0	2.3	+9.0	2.2			+18.3
σ_{n-1}	1.3	25.8	1.1	21.3	1.3	34.9	1.0			45.7

TABLE 19

Pulmonary Function Response to Placebo Vs. No Drug: FVC, FEV_{1.0}, PEFR

Five-Minutes Post-Exercise

Subject	FVC Placebo (liter)	Δ FVC Placebo ($\Delta\%$)	FVC No Drug (liter)	Δ FVC No Drug ($\Delta\%$)	FEV _{1.0} Placebo (liter)	Δ FEV _{1.0} Placebo ($\Delta\%$)	FEV _{1.0} No Drug (liter)	Δ FEV _{1.0} No Drug ($\Delta\%$)	PEFR Placebo (liter/sec)	Δ PEFR Placebo ($\Delta\%$)	PEFR No Drug (liter/sec)	Δ PEFR No Drug ($\Delta\%$)
1	3.3	-5.7	3.8	-2.6	2.5	-10.7	2.9	-9.4	6.0	-4.8	6.9	-5.5
2	3.9	-22.0	4.4	+12.0	2.1	-25.0	2.5	-13.8	3.9	-29.1	5.0	-12.3
3	3.3	-26.7	4.3	-10.4	1.4	-36.4	2.0	-16.7	3.8	-22.4	4.2	-22.2
4	2.5	-26.5	2.6	-23.5	1.5	-37.5	1.5	-31.8	3.0	-42.3	3.0	-33.3
5	5.2	0.0	4.7	-13.0	2.9	0.0	2.5	-19.4	6.6	+4.8	5.8	-12.1
6	3.6	+2.9	3.5	-5.4	2.8	0.0	2.8	-3.4	5.7	-3.4	5.8	-4.9
7	3.0	-3.2	3.2	-8.6	2.9	0.0	3.1	-3.1	5.7	-5.0	5.9	-3.3
8	3.3	-5.7	2.6	-7.1	2.0	-4.8	1.4	-12.5	4.4	+2.3	3.2	-5.9
9	3.0	-11.8	3.4	-5.5	2.3	-14.8	2.3	-20.7	5.4	-11.5	5.3	-14.5
10	5.7	-1.7	6.2	-3.1	4.3	+2.4	4.5	-6.3	9.1	0.0	9.8	-7.5
11	2.2	-31.3	2.6	-16.1	1.3	-51.9	1.4	-26.3	2.9	-55.4	2.9	-29.3
12	3.1	-18.4	3.1	-16.2	1.7	-32.0	2.0	-23.1	4.0	-29.8	4.2	-23.6
\bar{x}	3.5	-12.5	3.7	-8.3	2.3	-17.6	2.4	-15.5	5.0	-16.4	5.2	-14.5
$\sigma n-1$	1.0	11.9	1.1	8.9	0.9	18.4	0.9	9.1	1.8	19.3	1.9	10.2

TABLE 20

Pulmonary Function Response to Placebo Vs. No Drug: MEF40%, MEF40% (P)

Five-Minute Post-Exercise

Subject	MEF40% Placebo (liter/Sec)	MEF40% Placebo (liter/Sec)		MEF40% No Drug (liter/Sec)		MEF40% Placebo (liter/Sec)		MEF40% No Drug (liter/Sec)		MEF40% No Drug (Δ%)	
		ΔMEF40% Placebo (Δ%)	MEF40% No Drug (liter/Sec)	ΔMEF40% No Drug (Δ%)	MEF40% Placebo (liter/Sec)	ΔMEF40% Placebo (Δ%)	MEF40% No Drug (liter/Sec)	ΔMEF40% No Drug (Δ%)	MEF40% No Drug (liter/Sec)	ΔMEF40% No Drug (Δ%)	
1	1.8	-28.0	2.4	-22.6	1.2	-42.9	1.4	-48.1			
2	0.5	-64.3	1.0	-33.3	0.3	-76.9	0.8	-38.5			
3	0.4	-36.4	0.7	-50.0	0.2	-80.0	0.5	-64.3			
4	0.6	-62.5	0.5	-64.3	0.6	-64.7	0.5	-64.3			
5	1.6	-11.1	1.0	-44.4	1.4	-22.2	0.7	-50.0			
6	2.8	0.0	2.3	-17.9	2.2	-15.4	1.4	-26.3			
7	3.5	-5.4	2.9	-31.0	3.5	-14.6	2.4	-40.0			
8	1.1	0.0	0.6	-25.0	1.0	0.0	0.4	-20.0			
9	1.5	-28.6	1.4	-30.0	1.3	-31.6	1.0	-50.0			
10	3.1	+10.7	2.9	-19.4	2.7	+22.7	2.5	-30.6			
11	0.2	-92.9	0.5	-50.0	0.2	-93.3	0.3	-57.1			
12	0.4	<u>-75.0</u>	<u>0.8</u>	<u>-57.9</u>	<u>0.4</u>	<u>-80.0</u>	<u>0.9</u>	<u>-67.9</u>			
X	1.5	-32.8	1.5	-37.2	1.3	-41.6	1.1	-46.4			
on-1	1.1	33.8	0.9	15.7	1.1	37.1	0.7	15.7			

TABLE 21

Pulmonary Function Response to Placebo Vs. No Drug: FVC, FEV_{1.0}, PEFRPost-Alupent

Subject	FVC Placebo (liter)	Δ FVC Placebo (Δ%)	FVC No Drug (liter)	Δ FVC No Drug (Δ%)	FEV _{1.0} Placebo (liter)	Δ FEV _{1.0} Placebo (Δ%)	FEV _{1.0} No Drug (liter)	Δ FEV _{1.0} No Drug (Δ%)	PEFR Placebo (liter/sec)	Δ PEFR Placebo (Δ%)	PEFR No Drug (liter/sec)	Δ PEFR No Drug (Δ%)
1	3.6	+2.9	4.0	+2.6	3.0	+7.1	3.3	+3.1	7.0	+11.1	8.2	+12.3
2	5.2	+4.0	5.3	+6.0	3.0	+7.1	3.4	+17.2	5.7	+3.6	6.2	+8.8
3	4.8	+6.7	5.7	+18.8	2.2	0.0	2.9	+20.8	4.8	-2.0	5.8	+7.4
4	3.6	+5.9	3.4	0.0	2.7	+12.5	2.3	+4.5	6.7	+28.8	5.6	+24.4
5	5.3	+3.9	5.5	+1.9	3.7	+27.6	3.8	+22.6	8.2	+30.2	8.4	+27.3
6	3.5	0.0	3.7	0.0	3.0	+7.1	3.1	+6.9	5.9	0.0	6.2	+1.6
7	3.2	+3.2	3.5	0.0	3.1	+6.9	3.3	+3.1	6.3	+5.0	6.6	+8.2
8	4.0	+14.3	3.6	+28.6	2.7	+28.6	2.2	+37.5	5.4	+25.6	4.6	+35.3
9	3.6	+5.9	3.8	+5.5	3.2	+18.5	3.2	+10.3	7.0	+14.8	6.7	+8.1
10	5.9	+1.7	6.3	-1.6	4.6	+9.5	5.0	+4.2	10.0	+9.9	10.4	-1.9
11	3.0	-6.3	3.1	0.0	2.4	-11.1	1.9	0.0	5.3	-18.5	4.9	+19.5
12	<u>3.2</u>	<u>-15.8</u>	<u>3.3</u>	<u>-10.8</u>	<u>2.0</u>	<u>-20.1</u>	<u>2.1</u>	<u>-19.2</u>	<u>4.2</u>	<u>-26.3</u>	<u>4.4</u>	<u>-20.1</u>
\bar{x}	4.1	+2.2	4.3	+4.3	3.0	+7.8	3.0	+9.3	6.4	+6.9	6.5	+10.9
on-1	1.0	7.4	1.1	10.2	0.7	14.0	0.9	14.1	1.6	17.5	1.8	14.7

TABLE 22

Pulmonary Function Response to Placebo Vs. No Drug: MEF40%, MEF40% (P)

Subject	MEF40% Placebo (liter/Sec)	Post-Alupent			Δ MEF40% (P) No Drug ($\Delta\%$)
		Δ MEF40% Placebo ($\Delta\%$)	MEF40% No Drug (liter/Sec)	Δ MEF40% (P) Placebo (liter/Sec)	
1	3.3	+32.0	3.8	+22.6	3.8
2	1.6	+14.3	2.0	+33.3	1.7
3	1.1	0.0	1.9	+35.7	1.2
4	2.2	+37.5	1.6	+14.3	2.6
5	2.9	+61.1	2.8	+55.6	3.0
6	3.2	+14.3	3.2	+14.3	3.7
7	4.8	+29.7	4.6	+9.5	4.7
8	2.1	+90.9	1.9	+137.5	2.6
9	3.5	+66.7	3.4	+70.0	4.2
10	4.1	+46.4	4.1	+13.9	5.7
11	1.8	-35.7	1.4	+40.0	2.4
12	<u>0.7</u>	<u>-56.3</u>	<u>1.1</u>	<u>+42.1</u>	<u>0.8</u>
\bar{x}	2.6	+25.1	2.7	+33.7	3.0
$\bar{m}-1$	1.2	41.8	1.2	42.9	1.5
					65.3
					1.2
					105.7
					105.7
					+71.9
					+54.6
					2.8

TABLE 23

* $\Delta(\Delta PFT's)$ For Placebo Vs. No Drug

Immediate Post-Exercise

Subject	$\Delta(\Delta FVC)$		$\Delta(\Delta FEV_1.0)$		$\Delta(\Delta PEF)$		$\Delta(\Delta MEF40\% (P))$	
	Placebo Vs. No Drug ($\Delta\%$)	No Drug ($\Delta\%$)	Placebo Vs. No Drug ($\Delta\%$)					
1	+5.7	+3.6		+7.5		+12.9		+7.9
2	-6.0	-6.9		-8.9		-13.3		-30.8
3	-14.8	-17.1		-13.4		-30.5		-30.0
4	+3.0	+1.1		-8.1		-9.9		-5.5
5	+3.9	+7.8		+2.7		-11.1		-31.8
6	+8.1	+7.1		+3.5		+17.9		+4.9
7	0.0	+3.8		+0.1		+28.3		+4.6
8	-7.8	-7.7		-2.5		-31.8		-90.0
9	-2.6	+11.1		+5.0		+28.1		+37.6
10	+8.1	+11.6		+11.6		+45.7		+51.9
11	-3.2	-16.4		-27.3		-41.4		-31.0
12	-5.3	-0.3		-8.5		-3.0		+0.7
\bar{x}	-0.9	-0.2		-3.2		-0.7		-9.3
$\sigma n-1$	7.0	9.8		10.7		27.3		37.1
	t=0.5	t=0.1		t=1.0		t=0.1		t=0.9

*Explanation of $\Delta(\Delta PFT)$ Notation: $\Delta(\Delta FVC)$ for Placebo Vs. No Drug $\Delta(\Delta FVC)$ Placebo - $\Delta(\Delta FVC)$ No Drug

TABLE 24

 $\Delta(\Delta\text{PFT}'s)$ For Placebo Vs. No Drug

Subject	Five-Minutes Post-Exercise						$\Delta(\Delta\text{MEF}40\%(\text{P}))$ Placebo Vs. No Drug ($\Delta\%$)	
	$\Delta(\Delta\text{FVC})$		$\Delta(\Delta\text{FEV}_1.0)$		$\Delta(\Delta\text{PEFR})$			
	Placebo ($\Delta\%$)	No Drug ($\Delta\%$)	Placebo ($\Delta\%$)	No Drug ($\Delta\%$)	Placebo ($\Delta\%$)	No Drug ($\Delta\%$)		
1	-3.1	-1.3	+0.7	-5.4	+5.2			
2	-34.0	-11.2	-16.8	-31.0	-38.4			
3	-16.3	-19.7	-0.2	+13.6	-15.0			
4	-3.0	-5.7	-9.0	+1.8	-0.4			
5	+13.0	-19.4	16.9	+33.3	+27.8			
6	+8.3	+3.4	+1.5	+17.9	+10.9			
7	+5.4	+3.1	-1.7	+25.6	+25.4			
8	+1.4	+7.7	+8.2	+25.0	+20.0			
9	-6.3	+5.9	+3.0	+1.4	+18.4			
10	+1.4	+8.7	+7.5	+30.1	+53.3			
11	-15.2	-25.6	-26.1	-42.9	-36.2			
12	-2.2	-8.4	-6.2	-17.1	-12.1			
X	-4.2	-2.0	-1.9	+4.4	+4.9			
on-1	12.7	12.8	11.5	24.7	27.0			
	t=1.2	t=0.5	t=0.6	t=0.6	t=0.6			

TABLE 25

$\Delta(\Delta PFT's)$ For Placebo Vs. No Drug

Subject	Post-Alupent			$\Delta(\Delta FEV_1)$			$\Delta(\Delta PEFR)$			$\Delta(\Delta MEF40\% (P))$		
	Placebo Vs. No Drug (Δ%)	Placebo Vs. No Drug (Δ%)	Placebo Vs. No Drug (Δ%)									
1	+0.3	+4.0	-1.2	-1.2	+9.4	+25.4	-	-	-	-	-	-
2	-2.0	-10.1	-5.2	-5.2	-19.0	-53.8	-	-	-	-	-	-
3	-12.1	-20.8	-9.4	-9.4	-35.7	-30.0	-	-	-	-	-	-
4	+5.9	+8.0	+4.9	+4.9	+23.2	+38.6	-	-	-	-	-	-
5	+2.0	+5.0	+2.9	+2.9	+5.5	-47.7	-	-	-	-	-	-
6	0.0	+0.2	-1.6	-1.6	0.0	-26.1	-	-	-	-	-	-
7	+3.2	+3.8	+3.2	+3.2	+20.2	+7.1	-	-	-	-	-	-
8	-14.3	-8.9	-9.7	-9.7	-46.6	-220.0	-	-	-	-	-	-
9	+0.4	+8.2	+6.7	+6.7	-3.2	+51.1	-	-	-	-	-	-
10	+3.3	+5.3	+11.8	+11.8	+32.5	+120.5	-	-	-	-	-	-
11	-6.3	-11.1	-38.0	-38.0	-75.7	-62.9	-	-	-	-	-	-
12	-5.0	-0.9	-6.2	-6.2	-14.2	-10.0	-	-	-	-	-	-
-	-	-12.1	-1.4	-4.1	-8.6	-17.3	-	-	-	-	-	-
\bar{x}	-	6.2	9.2	12.5	31.5	82.5	-	-	-	-	-	-
on-1	-	t=1.1	t=0.5	t=1.1	t=0.5	t=0.7	-	-	-	-	-	-

TABLE 26

Pulmonary Function Response to Vitamin C Vs. Placebo: FVC, FEV_{1.0}, PEFR

Subject	Before Exercise					
	FVC Vitamin C (liter)	FVC Placebo (liter)	FEV _{1.0} Vitamin C (liter)	FEV _{1.0} Placebo (liter)	PEFR Vitamin C (liter/sec)	PEFR Placebo (liter/sec)
1	3.7	3.5	2.8	2.8	6.1	6.3
2	5.1	5.0	3.0	2.8	5.7	5.5
3	4.3	4.5	2.0	2.2	4.3	4.9
4	3.4	3.4	2.1	2.4	4.1	5.2
5	4.5	5.1	2.4	2.9	6.3	6.3
6	3.5	3.5	2.7	2.8	5.2	5.9
7	2.7	3.1	2.3	2.9	5.3	6.0
8	3.1	3.5	1.8	2.1	3.9	4.3
9	3.2	3.4	2.5	2.7	5.4	6.1
10	6.1	5.8	4.4	4.2	9.7	9.1
11	2.9	3.2	2.1	2.7	5.1	6.5
12	3.7	3.8	2.5	2.5	5.6	5.7
	3.9	4.0	2.6	2.8	5.6	6.0
\bar{x}	1.0	0.9	0.7	0.5	1.5	1.2

TABLE 27

Pulmonary Function Response to Vitamin C Vs. Placebo: MEF40%, MEF40% (P), Vmax50%

Subject	Before Exercise					
	MEF40% Vitamin C (liter/Sec)	MEF40% Placebo (liter/Sec)	MEF40% (P) Vitamin C (liter/Sec)	MEF40% (P) Placebo (liter/Sec)	Vmax50% Placebo (liter/Sec)	Vmax50% Vitamin C (liter/Sec)
1	2.2	2.5	1.7	2.1	3.0	3.4
2	1.4	1.4	1.2	1.3	1.9	1.9
3	0.9	1.1	0.8	1.0	1.2	1.4
4	1.2	1.6	1.2	1.7	1.6	1.8
5	1.3	1.8	1.1	1.8	1.7	2.2
6	2.3	2.8	1.5	2.6	3.1	3.6
7	2.3	3.7	2.4	4.1	2.9	4.6
8	1.0	1.1	0.9	1.0	1.3	1.5
9	1.8	2.1	1.8	1.9	2.1	2.7
10	2.9	2.8	3.0	2.2	3.9	3.9
11	1.6	2.8	1.8	3.0	2.2	3.6
12	1.8	1.6	2.4	2.0	2.3	2.1
-						
X	1.7	2.1	1.7	2.1	2.3	2.7
σn-1	0.6	0.8	0.7	0.9	0.8	1.1

Pulmonary Function Response to Vitamin C Vs. Placebo: FVC, FEV_{1.0}, PEFR Table 28

Subject	FVC Vitamin C (liter)	Δ FVC Vitamin C ($\Delta\%$)	FVC Placebo (liter)	Δ FVC Placebo ($\Delta\%$)	FEV _{1.0} Vitamin C (liter)	Δ FEV _{1.0} Vitamin C ($\Delta\%$)	FEV _{1.0} Placebo (liter)	Δ FEV _{1.0} Placebo ($\Delta\%$)	PEFR		Δ PEFR Placebo ($\Delta\%$)	PEFR Placebo (liter/sec)
									Immediate	Post-Exercise		
1	3.5	-5.4	3.7	+5.7	2.7	-3.6	2.9	+3.6	6.5	+6.6	7.2	+14.3
2	4.8	-5.9	4.8	-4.0	3.2	-6.7	2.8	0.0	5.7	0.0	5.3	-3.6
3	4.7	+9.3	4.3	-4.4	2.2	+10.0	2.1	-4.5	5.0	+16.3	4.7	-4.1
4	3.3	-3.0	3.1	-8.8	2.3	+9.5	2.1	-12.5	4.5	+9.8	4.2	-19.2
5	4.7	+4.4	5.2	+2.0	2.8	+16.7	3.5	+20.7	7.7	+22.2	7.9	+25.4
6	3.3	-5.7	3.5	0.0	2.7	0.0	3.0	+7.1	5.4	+3.8	6.2	+5.1
7	2.8	+3.7	3.1	0.0	2.6	+13.0	3.1	+6.9	5.6	+5.7	6.3	+5.0
8	3.4	+9.7	3.4	+2.9	2.2	+22.2	2.2	+4.8	4.8	+23.1	4.7	+9.3
9	3.5	+9.4	3.5	+2.9	2.9	+16.0	3.0	+11.1	6.4	+18.5	6.6	+8.2
10	6.2	+1.6	6.0	+3.4	4.6	+4.5	4.6	+9.5	10.0	+3.1	9.9	+8.8
11	3.3	+13.8	3.2	0.0	2.5	+19.0	2.4	-11.1	6.0	+17.6	5.2	-20.0
12	3.9	+5.4	3.7	-2.6	2.4	-4.0	2.3	-8.0	5.2	-7.1	4.8	-15.8
\bar{x}	4.0	+3.1	4.0	-0.2	2.8	+8.1	2.8	+2.3	6.1	+10.0	6.1	+1.1
σn	1.0	6.8	0.9	4.1	0.7	9.8	0.7	9.9	1.5	9.5	1.6	14.0

TABLE 29

Pulmonary Function Response to Vitamin C Vs. Placebo: MEF40%, MEF40%, (P)

Subject	MEF40% Vitamin C (liter/sec)	Immediate Post-Exercise		MEF40% (P) Placebo (liter/sec)		MEF40% (P) Vitamin C (liter/sec)		MEF40% (P) Placebo (liter/sec)		ΔMEF40% (P) Placebo (Δ%)	
		ΔMEF40% Vitamin C (Δ%)	MEF40% Placebo (liter/sec)	ΔMEF40% Placebo (Δ%)	MEF40% (P) Vitamin C (Δ%)	MEF40% (P) Placebo (liter/sec)	ΔMEF40% (P) Placebo (Δ%)	ΔMEF40% (P) Placebo (liter/sec)	ΔMEF40% (P) Placebo (Δ%)	+9.0	34.9
1	2.1	-4.5	2.9	+16.0	1.7	0.0	2.5	+19.0			
2	1.3	-7.1	1.4	0.0	1.3	+8.3	1.2	-7.7			
3	1.2	+33.3	1.0	-9.1	0.8	0.0	0.7	-30.0			
4	1.5	+25.0	1.1	-31.3	1.4	+16.7	1.0	-41.2			
5	2.0	+53.8	2.4	+33.3	1.9	+72.7	1.4	+61.1			
6	2.3	0.0	3.3	+17.9	2.1	+40.0	3.0	+15.4			
7	3.5	+52.2	5.1	+37.8	4.1	+70.8	4.8	+17.1			
8	1.5	+50.0	1.3	+18.2	1.7	+88.9	1.3	+30.0			
9	2.6	+44.4	2.9	+38.1	3.1	+72.2	2.9	+52.6			
10	3.2	+10.3	4.0	+42.9	3.4	+13.3	3.9	+43.6			
11	2.9	+81.3	2.2	-21.4	3.4	+88.9	2.5	-16.7			
12	1.6	-11.1	1.3	-18.8	1.7	-29.2	1.3	-35.0			
\bar{x}	2.2	+27.3	2.4	+10.3	2.2	+36.9	2.2	+9.0			
σ_{n-1}	0.9	29.8	1.3	25.8	1.0	40.4	1.3	34.9			

TABLE 30

Pulmonary Function Response to Vitamin C Vs. Placebo: FVC, FEV _{1.0} , PEFR											
Five-Minute Post-Exercise											
	Vitamin C Subject (liter)	FVC Vitamin C (liter)	FVC Placebo (liter)	Δ FVC Placebo ($\Delta\%$)	FEV _{1.0} Vitamin C (liter)	FEV _{1.0} Placebo (liter)	Δ FEV _{1.0} Placebo ($\Delta\%$)	PEFR Vitamin C (liter/sec)	PEFR Placebo (liter/sec)	Δ PEFR Placebo ($\Delta\%$)	PEFR Vitamin C (liter/sec)
1	3.7	0.0	3.3	-5.7	2.6	-7.1	2.5	-10.7	5.8	-4.9	6.0
2	4.4	-13.7	3.9	-22.0	2.6	-13.3	2.1	-25.0	4.8	-15.8	3.9
3	3.8	-11.6	3.3	-26.7	1.6	-20.0	1.4	-36.4	3.7	-14.0	3.8
4	3.3	-3.0	2.5	-26.5	2.0	-4.8	1.5	-37.5	4.3	+4.9	3.0
5	4.8	+6.7	5.2	0.0	2.4	0.0	2.9	0.0	5.5	-19.0	6.6
6	3.4	-2.9	3.6	+2.9	2.4	-11.1	2.8	0.0	5.0	-3.8	5.7
7	2.5	-7.4	3.0	-3.2	2.2	-4.3	2.9	0.0	5.0	-5.7	5.7
8	2.9	-6.5	3.3	-5.7	1.8	0.0	2.0	-4.8	3.9	0.0	4.4
9	3.1	-3.1	3.0	-11.8	2.3	-8.0	2.3	-14.8	5.4	0.0	5.4
10	5.9	-3.3	5.7	-1.7	4.4	0.0	4.3	+2.4	9.5	-2.1	9.1
11	2.3	-20.7	2.1	-31.3	1.4	-33.3	1.3	-51.9	2.9	-41.2	2.9
12	3.3	<u>-10.8</u>	<u>3.1</u>	<u>-18.4</u>	<u>2.0</u>	<u>-20.0</u>	<u>1.7</u>	<u>-32.0</u>	<u>4.1</u>	<u>-26.8</u>	<u>4.0</u>
											<u>-29.8</u>
	\bar{x}	3.6	-6.4	3.5	-12.5	2.3	-10.2	2.3	-17.6	5.0	-10.7
SD	1.0	7.1	1.0	11.9	0.8	10.1	0.9	18.4	1.7	13.3	1.8

Pulmonary Function Response to Vitamin C Vs. Placebo: MEF40%, MEF40%(P) TABLE 31

Five-Minute Post-Exercise

Subject	MEF40% Vitamin C (liter/sec)	Δ MEF40% Vitamin C (Δ %)	MEF40% Placebo (liter/sec)	Δ MEF40% Placebo (Δ %)	MEF40% Vitamin C (liter/sec)	Δ MEF40% Vitamin C (Δ %)	MEF40% Placebo (liter/sec)	Δ MEF40% Placebo (Δ %)
1	1.7	-22.7	1.8	-28.0	0.9	-47.9	1.2	-42.9
2	1.0	-28.6	0.5	-64.3	0.7	-41.7	0.3	-76.9
3	0.6	-33.3	0.4	-36.4	0.4	-50.0	0.2	-80.0
4	1.2	0.0	0.6	-62.5	1.2	0.0	0.6	-64.7
5	1.3	0.0	1.6	-11.1	0.7	-36.4	1.4	-22.2
6	1.5	-34.8	2.8	0.0	0.8	-46.7	2.2	-15.4
7	1.7	-26.1	3.5	-5.4	2.2	-8.3	3.5	-14.6
8	0.9	-10.0	1.1	0.0	0.8	-11.1	1.0	0.0
9	1.6	-11.1	1.5	-28.6	1.4	-22.2	1.3	-31.6
10	2.6	-10.3	3.1	+10.7	2.5	-16.7	2.7	+22.7
11	0.5	-68.8	0.2	-92.9	0.6	-66.7	0.2	-93.3
12	0.9	-50.0	0.4	-75.0	0.8	-66.7	0.4	-80.0
\bar{x}	1.3	-24.6	1.5	-32.8	1.1	-34.5	1.3	-41.6
σ_{n-1}	0.6	20.5	1.1	33.8	0.6	22.5	1.1	37.1

TABLE 32

Pulmonary Function Response to Vitamin C Vs. placebo: FVC, FEV₁, PEFR

TABLE 33

Pulmonary Function Response to Vitamin C Vs. Placebo: MEF40%, MEF40% (P)
Post-Alupent

Subject	MEF40% (liter/sec)	Vitamin C (Δ%)	ΔMEF40% Vitamin C (Δ%)	MEF40% Placebo (liter/sec)	ΔMEF40% Placebo (Δ%)	MEF40% (P) Vitamin C (Δ%)	ΔMEF40% (P) Placebo (liter/sec)	ΔMEF40% (P) Placebo (Δ%)
1	3.1	+40.9	3.3	+32.0	2.8	+52.9	3.8	+81.0
2	2.0	+42.9	1.6	+14.3	2.2	+83.3	1.7	+30.8
3	1.3	+44.0	1.1	0.0	1.5	+87.5	1.2	+20.0
4	1.9	+58.3	2.2	+37.5	2.4	+100.0	2.6	+52.9
5	3.6	+176.9	2.9	+61.1	3.8	+245.5	3.0	+66.6
6	2.9	+26.1	3.2	+14.3	2.3	+53.3	3.7	+42.3
7	4.6	+100.0	4.8	+29.7	4.6	+91.7	4.7	+14.6
8	2.1	+110.0	2.1	+90.9	2.7	+200.0	2.6	+160.0
9	3.3	+83.3	3.5	+66.7	4.4	+144.4	4.2	+121.1
10	3.7	+27.6	4.1	+46.4	4.7	+56.7	5.7	+145.5
11	1.9	+18.8	1.8	-35.7	2.4	+33.3	2.4	-20.0
12	1.2	-33.3	0.7	-56.3	1.0	-58.3	0.8	-6.0
\bar{x}	2.6	+58.0	2.6	+25.1	2.9	+90.9	3.0	+54.6
$\sigma n-1$	1.1	53.7	1.2	41.8	1.2	78.9	1.5	65.3

TABLE 34

 $\Delta(\Delta\text{PFT}'s)$ For Vitamin C Vs. Placebo

Immediate Post-Exercise

Subject	$\Delta(\Delta\text{FVC})$			$\Delta(\Delta\text{PEFR})$			$\Delta(\Delta\text{MEF40%})$			$\Delta(\Delta\text{MEF40%}(P))$		
	Vitamin C (Δ%)	Vs.	Placebo	Vitamin C (Δ%)	Vs.	Placebo	Vitamin C (Δ%)	Vs.	Placebo	Vitamin C (Δ%)	Vs.	Placebo (Δ%)
1	-11.1			-7.2			+7.7			-20.5		-19.0
2	-1.9			-6.7			+3.6			-7.1		+16.0
3	+13.7			+14.5			+20.4			+42.4		+30.0
4	+5.8			+22.0			+29.0			+56.3		+57.9
5	+2.4			-4.0			-3.2			+20.5		+11.6
6	-5.7			-7.1			-1.3			-17.9		+24.6
7	+3.7			+6.1			+0.7			+14.4		+53.7
8	+6.8			+17.4			+13.3			+31.8		+58.9
9	+6.5			+4.9			+10.3			+6.3		+19.6
10	-1.8			-5.0			-5.7			-32.1		-30.3
11	+13.8			+30.1			+37.6			+102.7		+105.6
12	+8.0			+4.0			+8.7			+7.7		+5.8
\bar{x}	+3.4			+5.8			+8.8			+17.0		+27.9
$\sigma n-1$	7.5			12.7			14.1			37.3		37.1
											$t=1.6$	$t=2.6$
											$p<.05$	$p<.05$

TABLE 35

Δ(PFT's) For Vitamin C vs. Placebo

Five-Minute Post-Exercise

TABLE 36

 $\Delta(\Delta PFT's)$ For Vitamin C Vs. PlaceboPost-Alupent

Subject	Vitamin C Vs. Placebo ($\Delta\%$)	<u>$\Delta(\Delta FVC)$</u>			<u>$\Delta(\Delta FEV_{1.0})$</u>			<u>$\Delta(\Delta PEFR)$</u>			<u>$\Delta(\Delta MEF40\% (P))$</u>		
		Vitamin C Vs. Placebo ($\Delta\%$)	Vitamin C Vs. Placebo ($\Delta\%$)	Vitamin C Vs. Placebo ($\Delta\%$)	Vitamin C Vs. Placebo ($\Delta\%$)	Vitamin C Vs. Placebo ($\Delta\%$)	Vitamin C Vs. Placebo ($\Delta\%$)	Vitamin C Vs. Placebo ($\Delta\%$)	Vitamin C Vs. Placebo ($\Delta\%$)				
1	-0.2	+3.6	+6.9	+6.9	+3.4	+18.3	+18.3	+22.4	+3.4	+44.4	+44.4	+28.1	
2	-0.1	+2.9	+2.9	+2.9	+2.4	+6.5	+6.5	+6.5	+2.4	+20.8	+20.8	+52.5	
3	+9.6	+65.0	+65.0	+65.0	+6.5	+34.9	+34.9	+34.9	+6.5	-3.2	-3.2	+67.5	
4	-8.9	+6.5	+6.5	+6.5	+10.5	-3.4	-3.4	-3.4	+10.5	+7.7	+7.7	+47.1	
5	+20.5	+34.9	+34.9	+34.9	+15.8	+15.8	+15.8	+15.8	+10.3	+115.8	+115.8	+178.9	
6	0.0	-3.4	-3.4	-3.4	+5.5	+5.5	+5.5	+5.5	+9.3	+11.8	+11.8	+11.0	
7	+0.5	+0.5	+0.5	+0.5	+1.9	+1.9	+1.9	+1.9	-3.1	+70.3	+70.3	+77.1	
8	+8.3	+8.3	+8.3	+8.3	+20.6	+9.9	+9.9	+9.9	+10.3	+19.1	+19.1	+40.0	
9	+6.6	+6.6	+6.6	+6.6	+13.1	+13.1	+13.1	+13.1	+6.6	+16.6	+16.6	+23.3	
10	+3.2	+3.2	+3.2	+3.2	+8.0	+8.0	+8.0	+8.0	+3.5	-18.8	-18.8	-88.8	
11	+9.9	+9.9	+9.9	+9.9	+13.1	+13.1	+13.1	+13.1	+34.2	+54.5	+54.5	+53.3	
12	+13.1	+13.1	+13.1	+13.1	+8.0	+8.0	+8.0	+8.0	+3.3	+23.0	+23.0	+1.7	
		+5.2	+14.3	+14.3	+9.4	+32.9	+32.9	+32.9	+9.4	+36.3	+36.3		
\bar{x}		7.7	18.9	18.9	10.9	34.7	34.7	34.7	10.9	64.3	64.3		
$\sigma_{\bar{x}}$		t=2.4	t=2.6	t=2.6	t=3.0	t=3.3	t=3.3	t=3.3	t=3.0	t=2.0	t=2.0		
		p<.05	p<.05	p<.05	p<.02	p<.01	p<.01	p<.01	p<.02	p<.01	p<.01		

TABLE 37

PAIRED t-TESTS FOR $\Delta(\Delta \text{ PFT}'s)$

Placebo vs. No Drug				Vitamin C vs. Placebo			
Subject	t Immediate Post Exercise	t Five-Minute Post-Exercise	t Post-Alupent	t Immediate Post-Exercise	t Five-Minute Post-Exercise	t Post-Exercise	t Alupent
$\Delta(\Delta \text{ FVC})$	0.5	1.2	1.2	1.6	2.5	2.4	p < .05
$\Delta(\Delta \text{ FEV}_{1.0})$	0.1	0.5	0.5	1.5	2.2	2.6	p < .05
$\Delta(\Delta \text{ PEFR})$	1.0	0.6	1.1	2.2	1.2	3.0	p < .05
$\Delta(\Delta \text{ MEF}40\%)$	0.1	0.6	1.0	1.6	1.0	3.3	p < .02
$\Delta(\Delta \text{ MEF}40\%(P))$	0.9	0.6	0.7	2.6	0.8	2.0	p < .05

Analysis of Variance of PFT's on Three Experimental Days

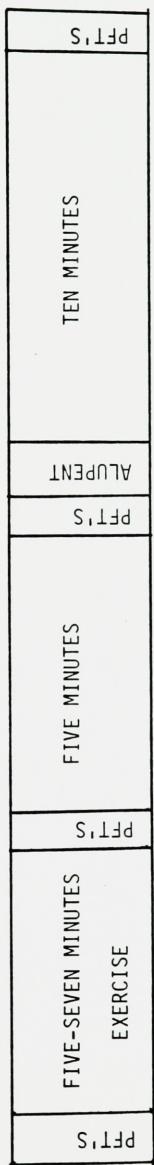
For Asthmatic Subjects

<u>PFT's</u>	<u>No Drug</u>	<u>Placebo</u>	<u>Vitamin C</u>	<u>F*</u>
Mean Baseline FVC (liter)	4.1	4.0	3.8	0.2
Mean Baseline FEV _{1.0} (liter)	2.8	2.8	2.6	0.2
Mean Baseline PEFR (liter/Sec)	6.0	6.0	5.6	0.3
Mean Baseline MEF40% (liter/Sec)	2.1	2.1	1.7	0.9
Mean Baseline MEF40% (P) (liter/Sec)	2.0	2.1	1.7	0.6
Mean Baseline Vmax50% (liter/Sec)	2.7	2.7	2.3	0.1

Critical F value for $p < .05 = 3.29$

SCHEMATIC REPRESENTATION OF DAILY ROUTINE

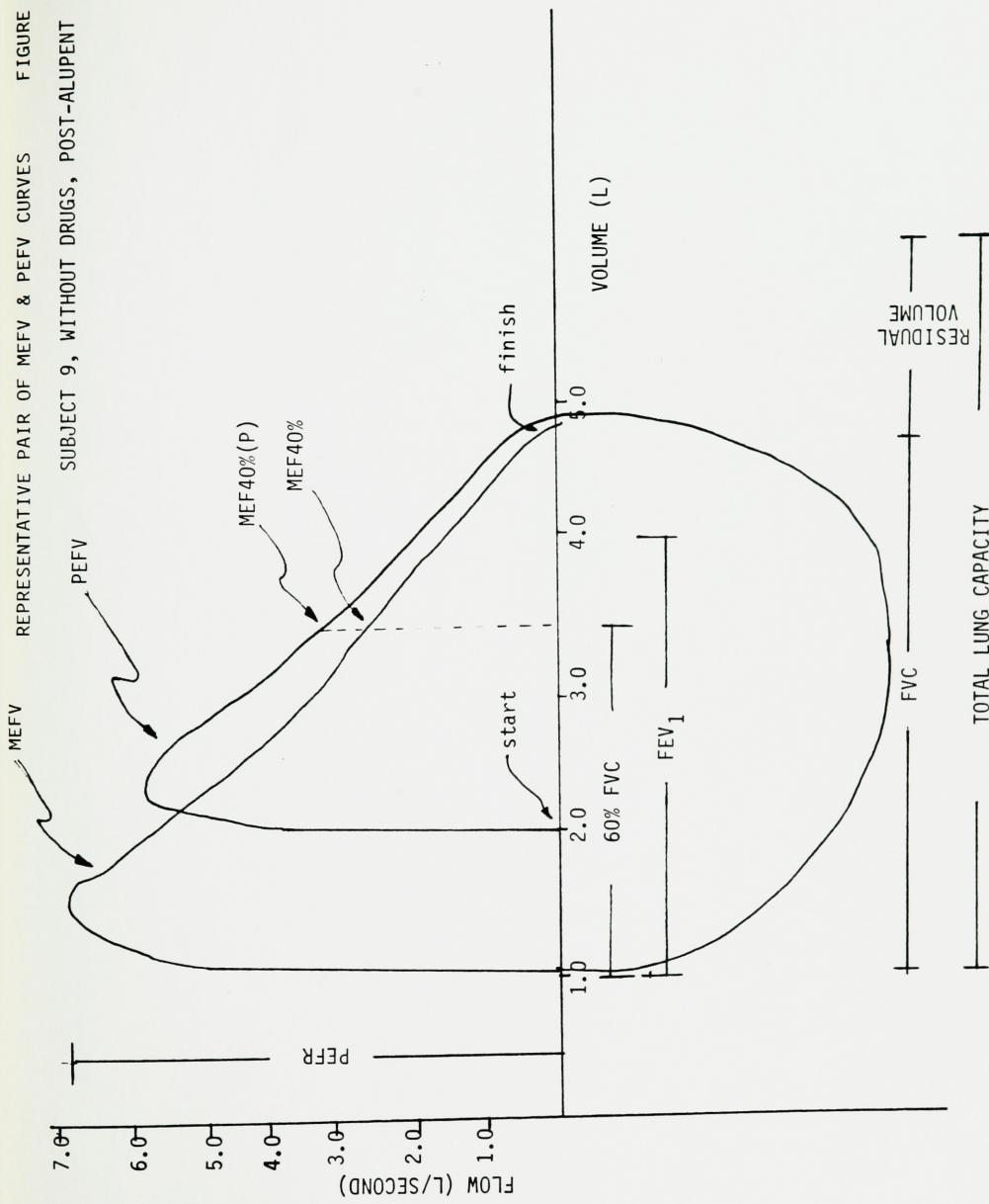
FIGURE 1



REPRESENTATIVE PAIR OF MEFV & PEFV CURVES

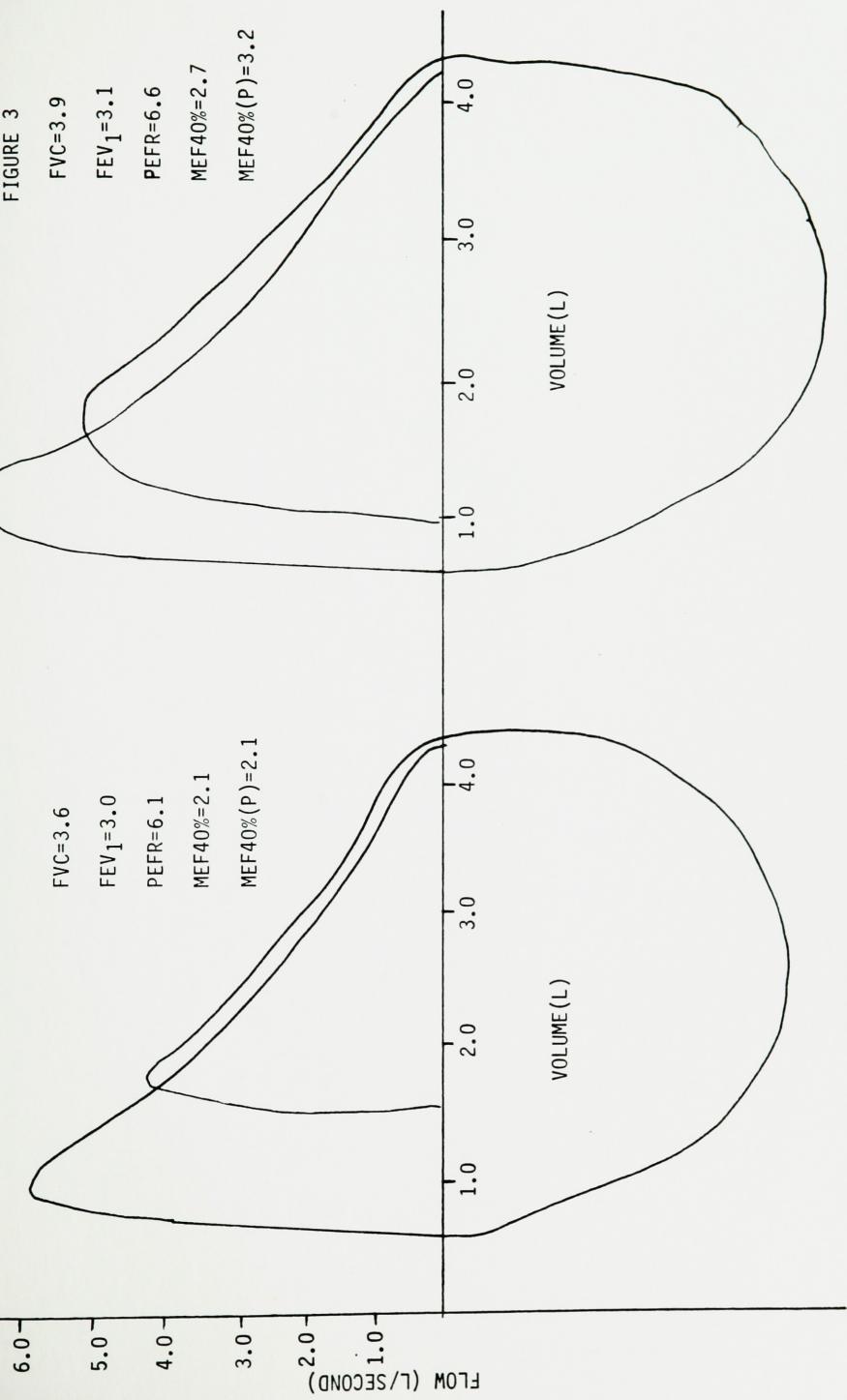
FIGURE 2

SUBJECT 9, WITHOUT DRUGS, POST-ALUPENT



REPRESENTATIVE MEFV & PEFV CURVES: SUBJECT 9, WITHOUT DRUGS

FIGURE 3

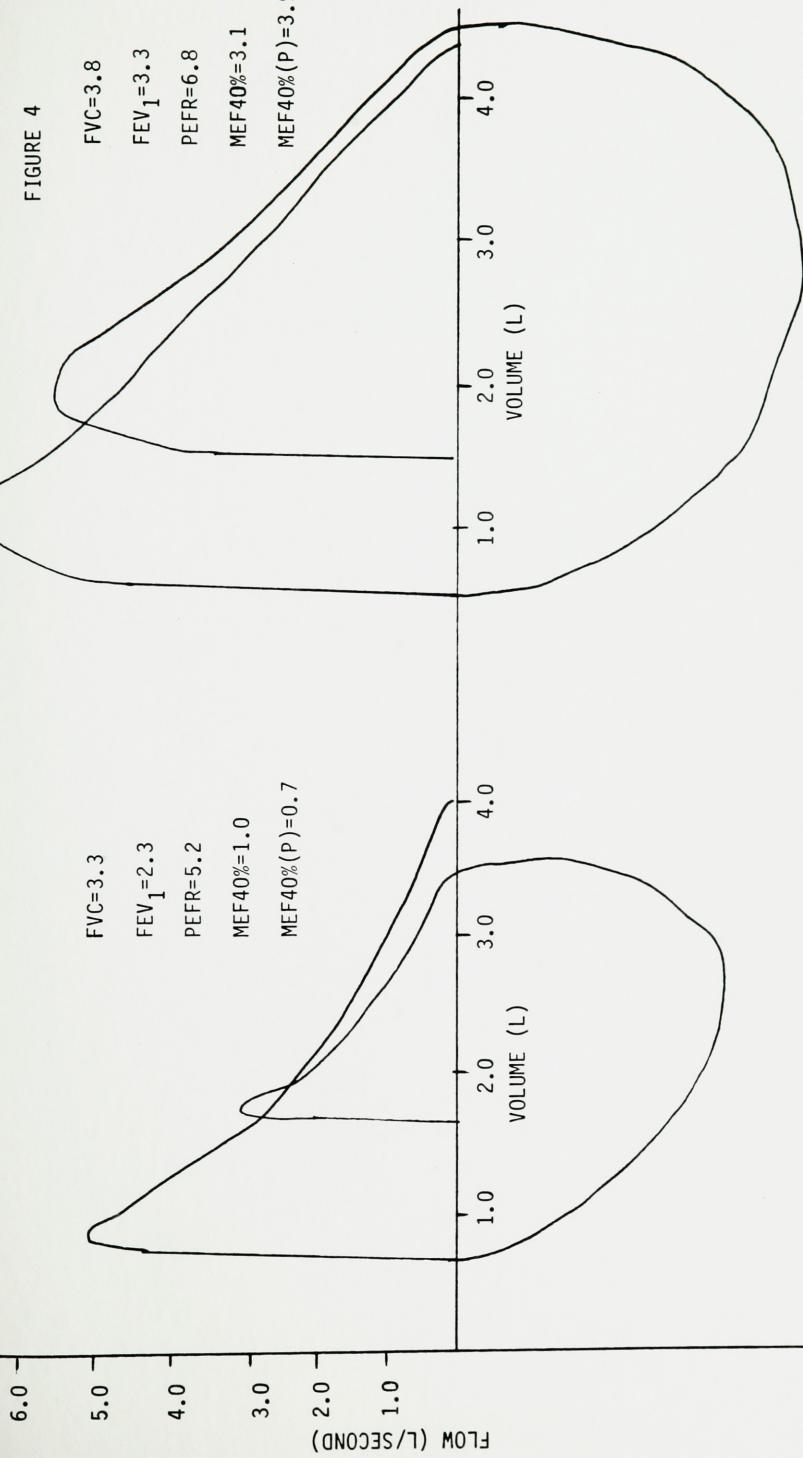


BASELINE

IMMEDIATE POST-EXERCISE

REPRESENTATIVE MEFV & PEFV CURVES: SUBJECT 9, WITHOUT DRUGS

FIGURE 4



FIVE-MINUTE POST-EXERCISE

POST-ALUPENT

PULMONARY FUNCTION RESPONSE TO EXERCISE WITHOUT DRUGS

FIGURE 5

FVC (MEAN \pm SEM)

FIVE-MINUTE
IMMEDIATELY
POST-EXERCISE

POST-ALUPENT

35

30

25

20

15

10

5

-5

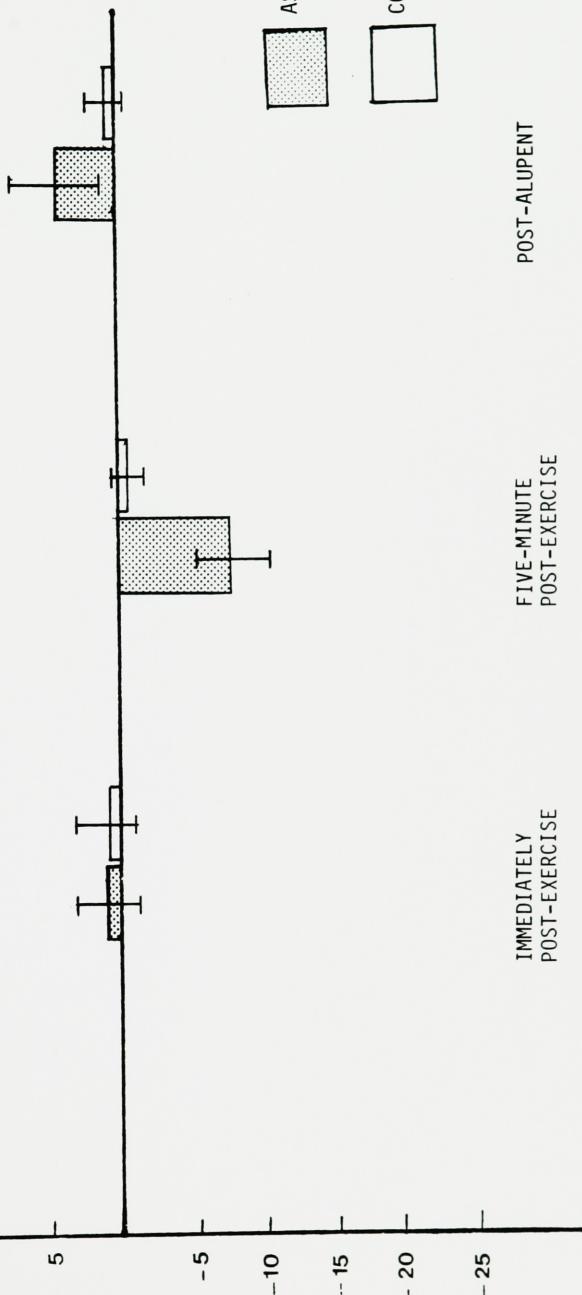
-10

-15

-20

-25

DELTA % BASELINE*

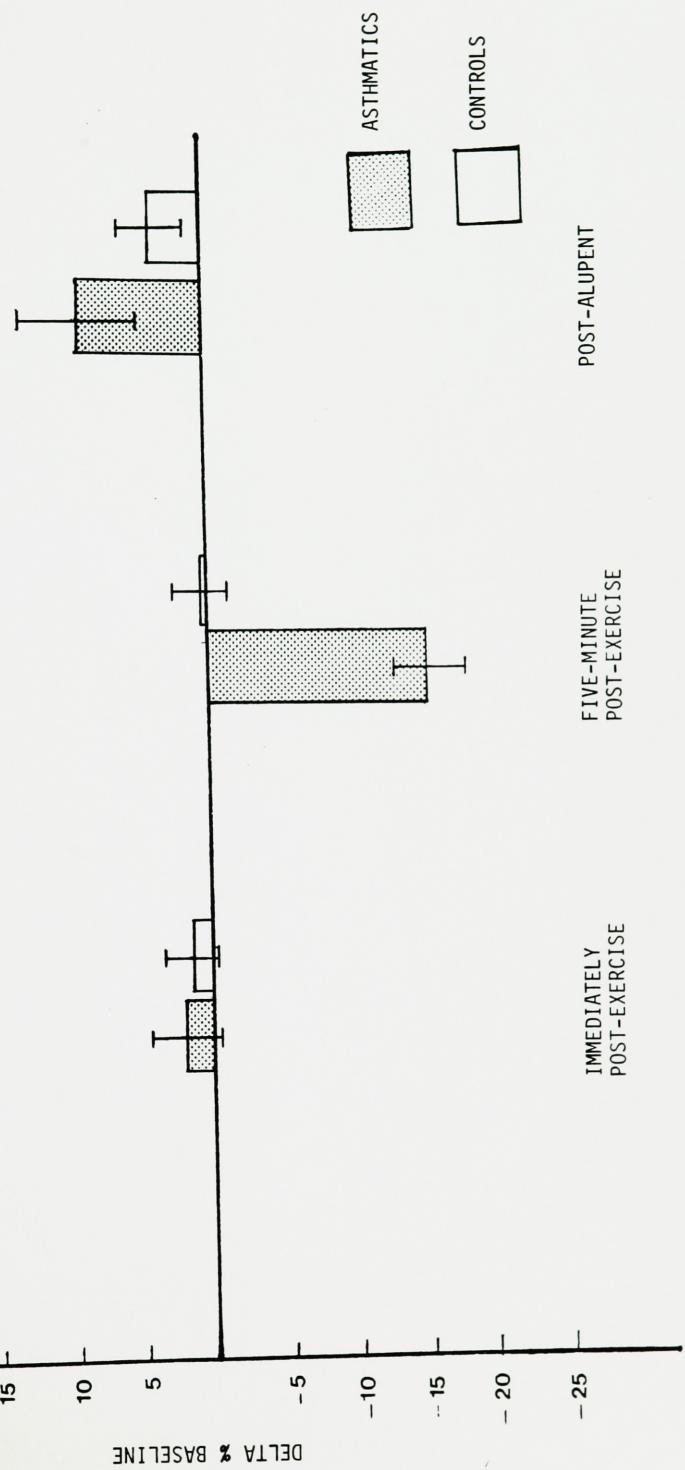


*DELTA % BASELINE=PERCENT CHANGE FROM BASELINE

PULMONARY FUNCTION RESPONSE TO EXERCISE WITHOUT DRUGS

FIGURE 6

FEV₁ (MEAN \pm SEM)



PULMONARY FUNCTION RESPONSE TO EXERCISE WITHOUT DRUGS
PEFR (MEAN \pm SEM)

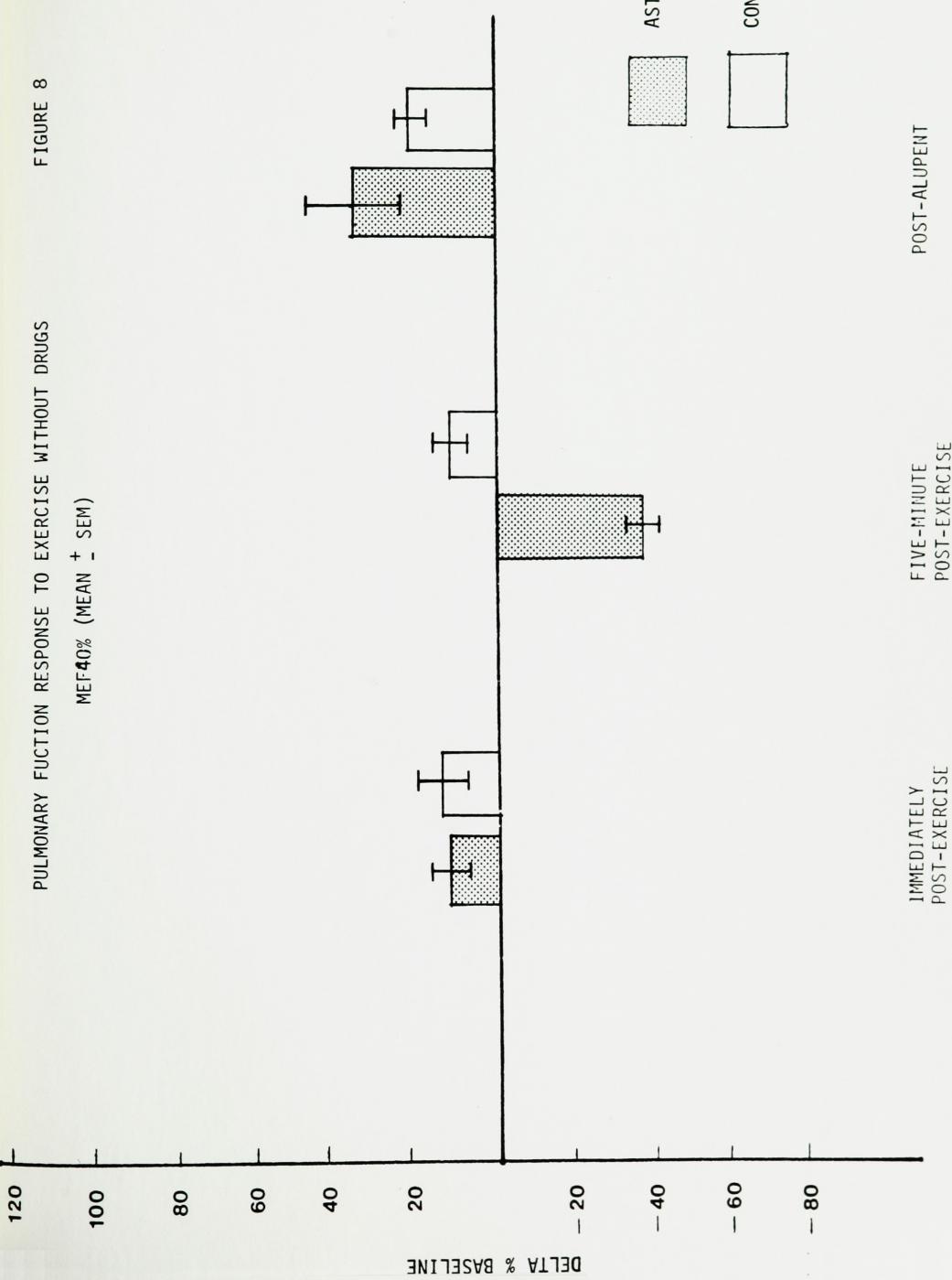
FIGURE 7



PULMONARY FUNCTION RESPONSE TO EXERCISE WITHOUT DRUGS

FIGURE 8

MEF_{40%} (MEAN \pm SEM)



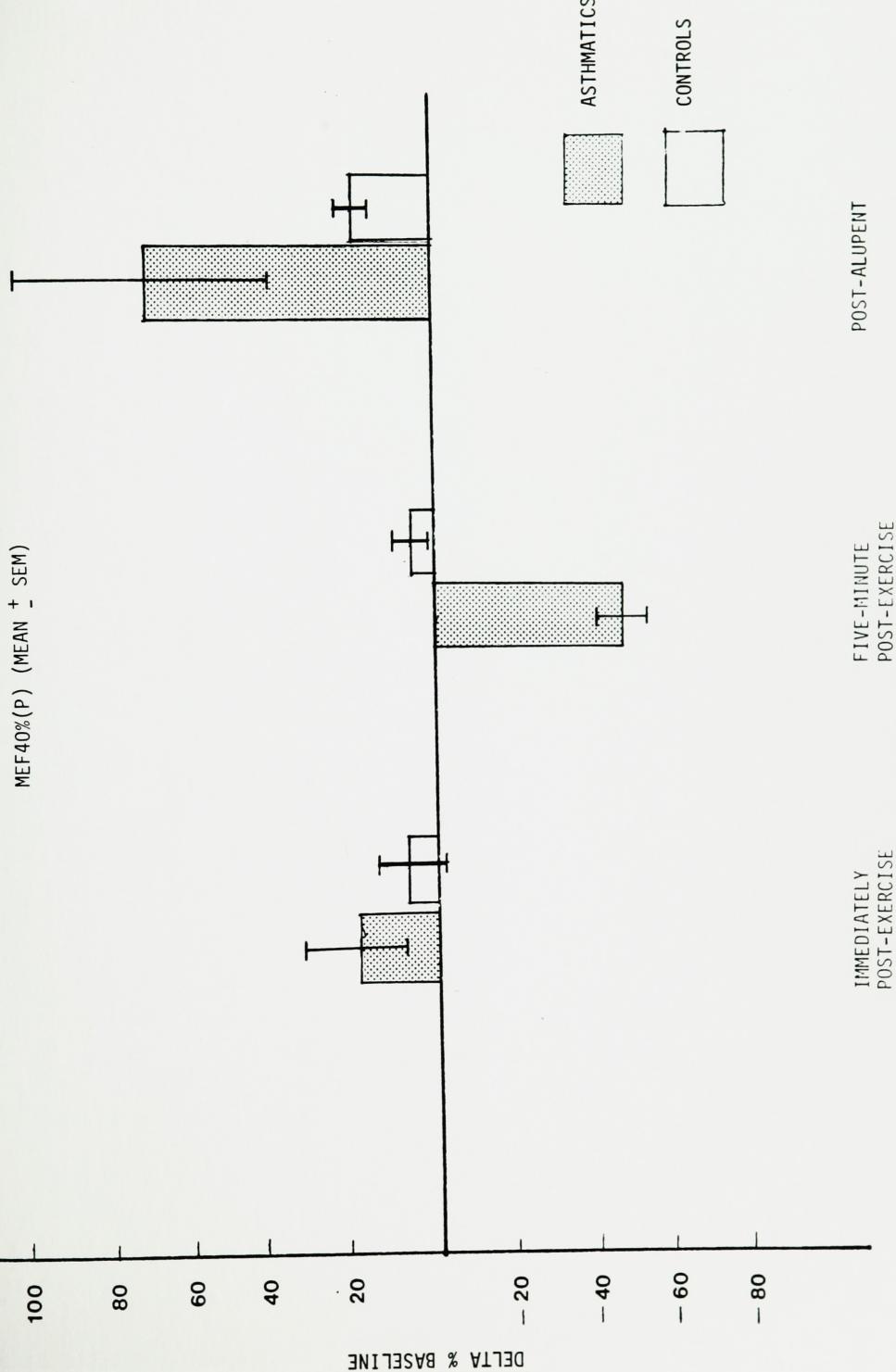
POST-ALUPENT

FIVE-MINUTE
POST-EXERCISE

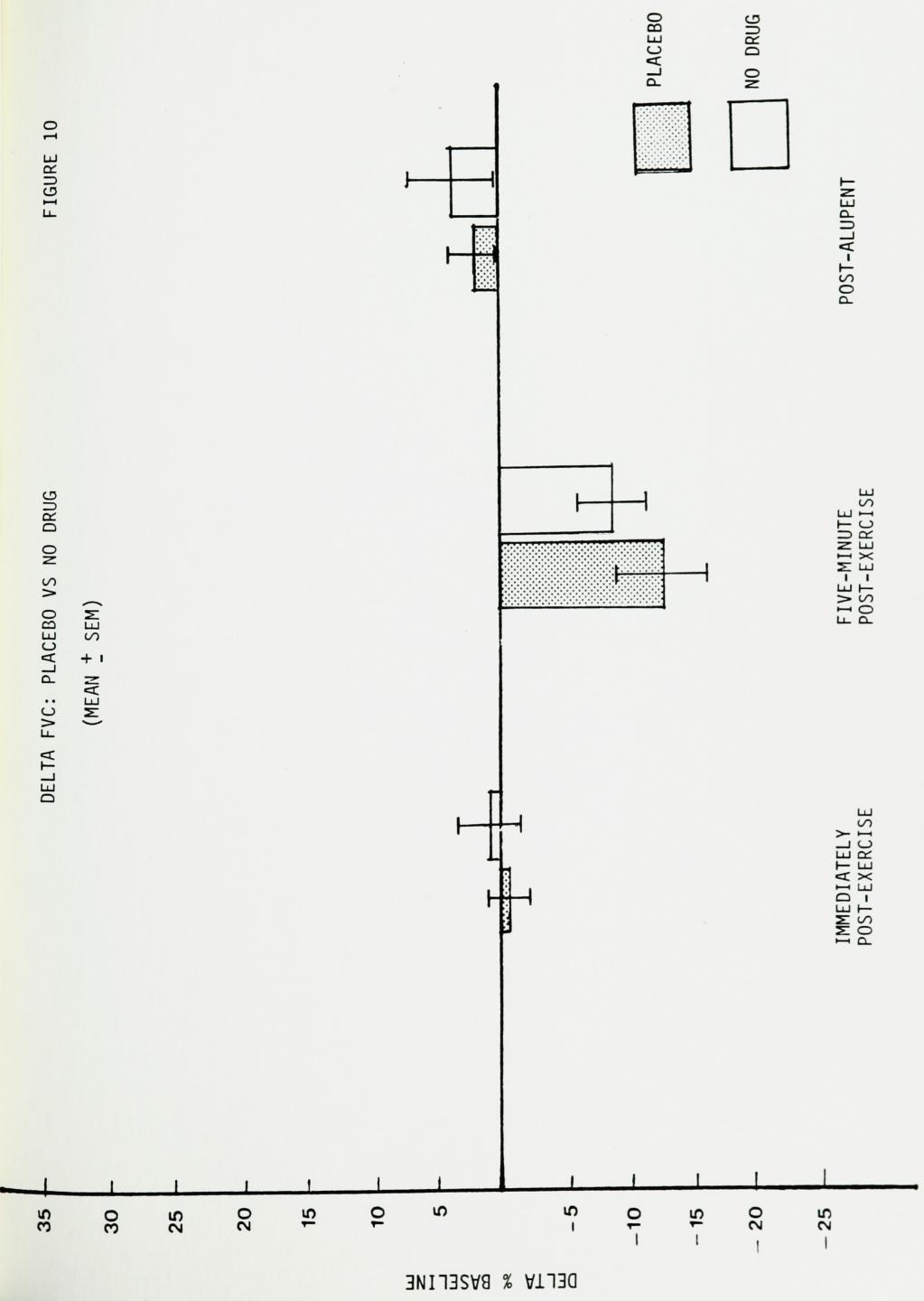
IMMEDIATELY
POST-EXERCISE

PULMONARY FUNCTION RESPONSE TO EXERCISE WITHOUT DRUGS

FIGURE 9



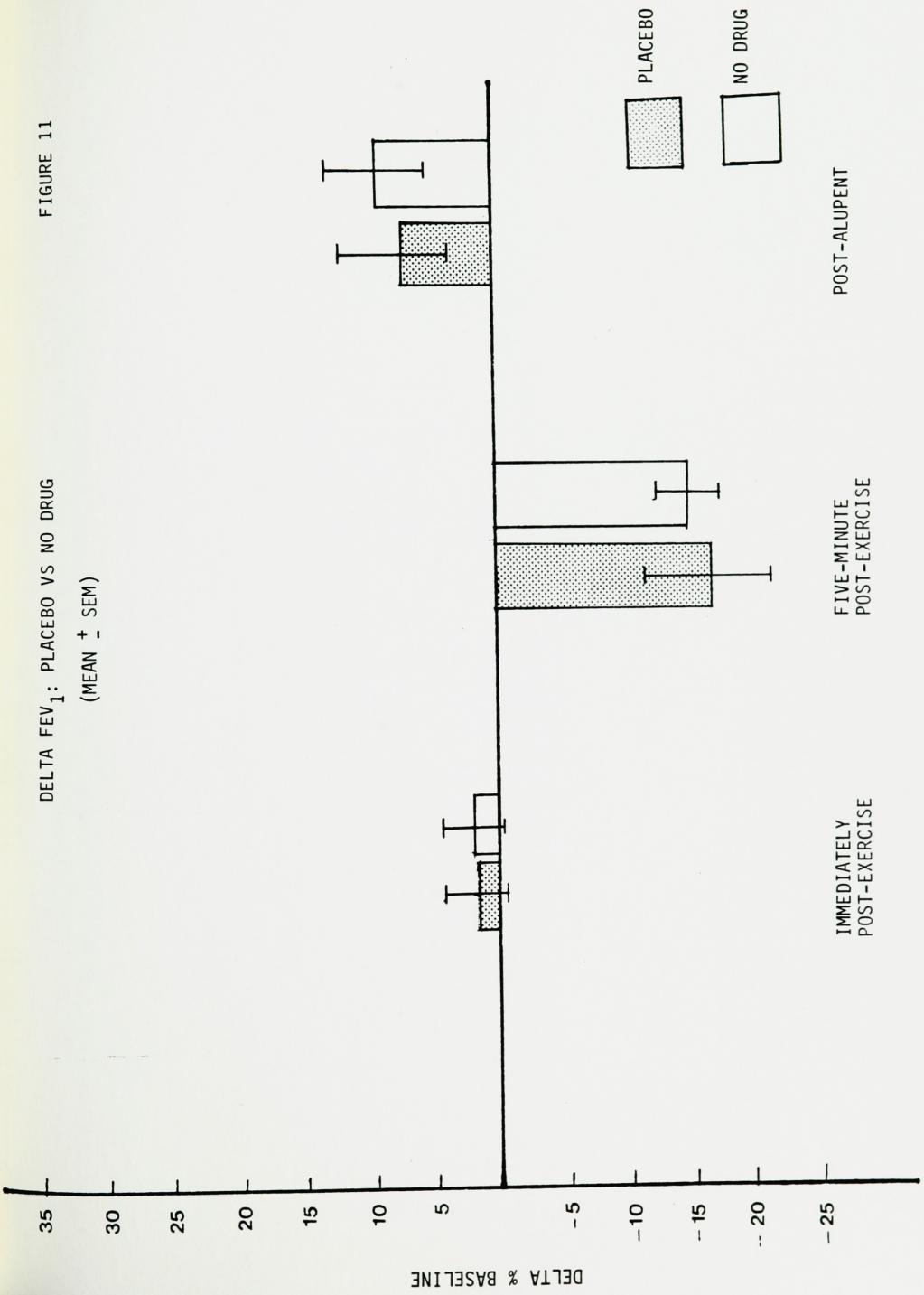
DELTA FVC: PLACEBO VS NO DRUG
(MEAN \pm SEM)



DELTA FEV₁: PLACEBO VS NO DRUG

(MEAN \pm SEM)

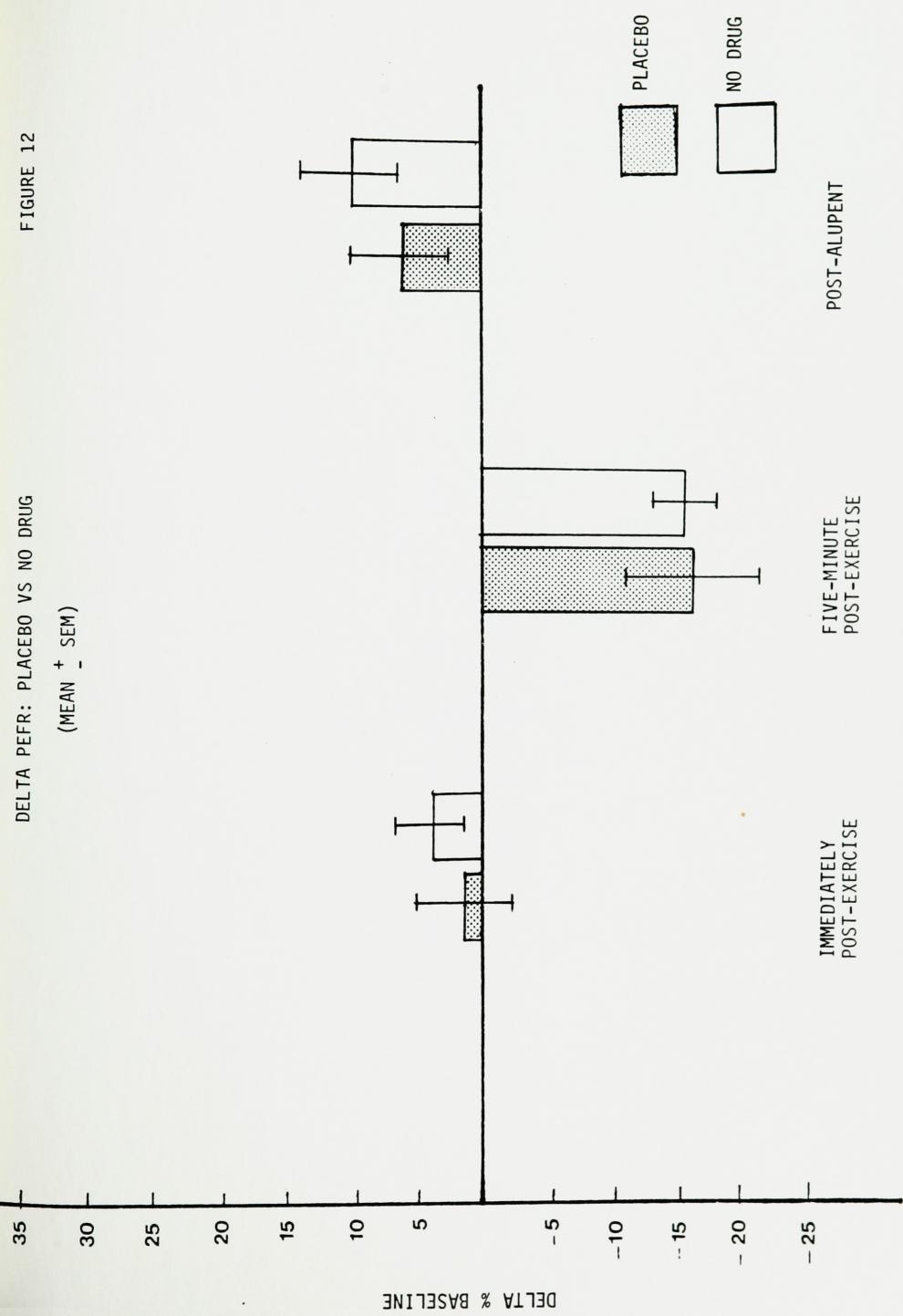
FIGURE 11



DELTA PEFR: PLACEBO VS NO DRUG

(MEAN + SEM)

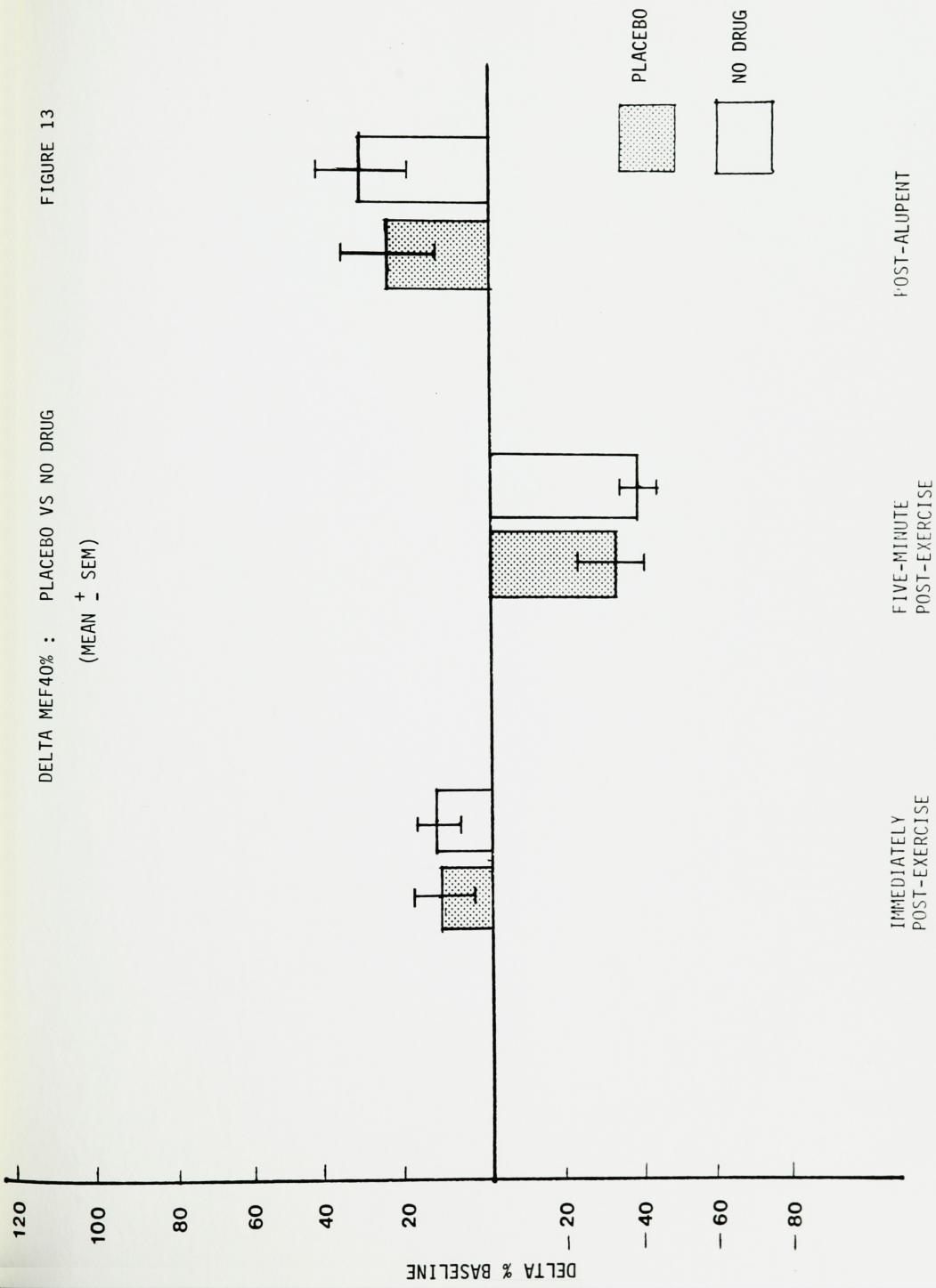
FIGURE 12



DELTA MEF40% : PLACEBO VS NO DRUG

(MEAN \pm SEM)

FIGURE 13



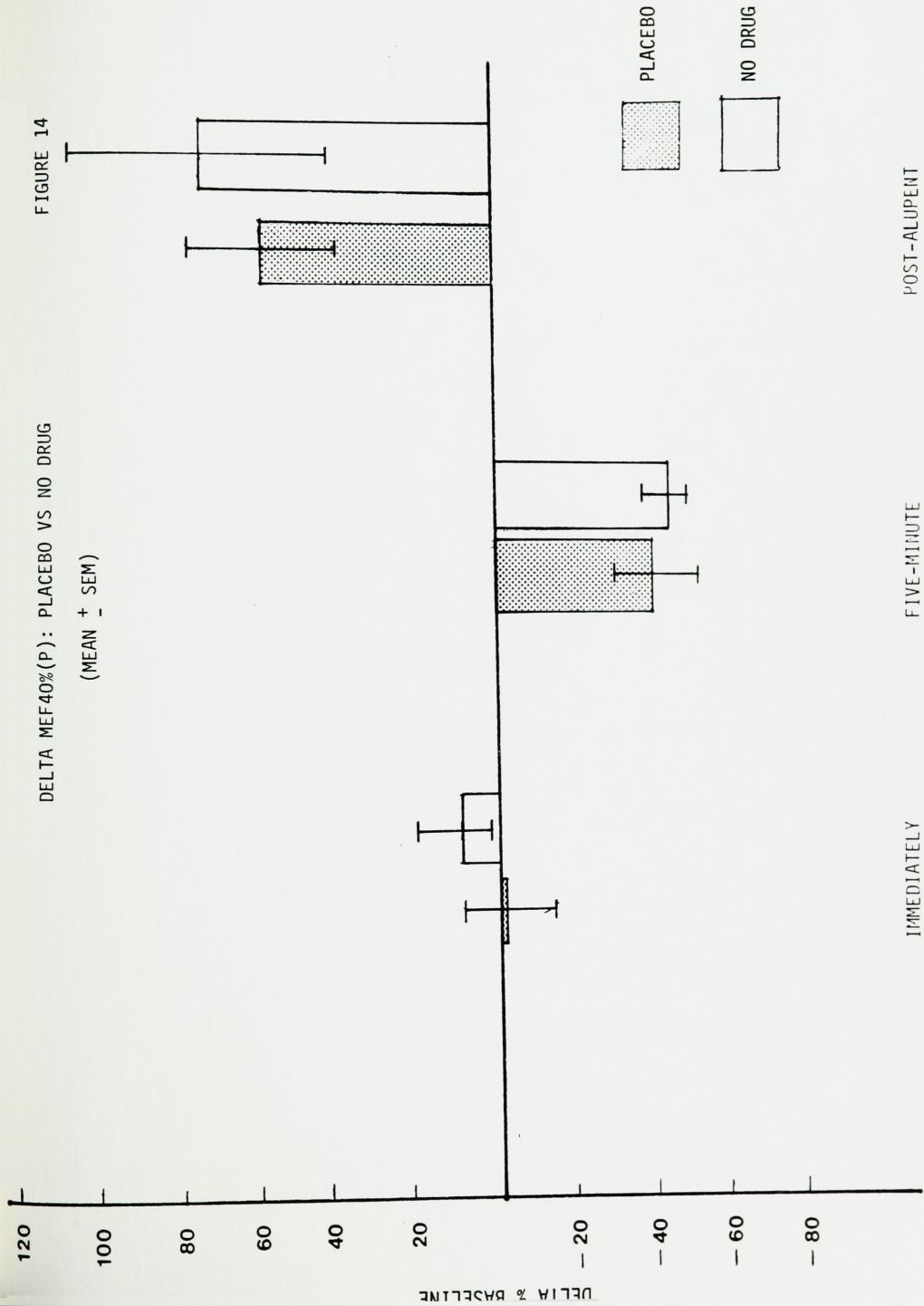
IMMEDIATELY
POST-EXERCISE

FIVE-MINUTE
POST-EXERCISE

POST-ALUPENT

FIGURE 14
DELTA MEF40% (P) : PLACEBO VS NO DRUG

(MEAN \pm SEM)



POST-ALUPENT

FIVE-MINUTE
POST-EXERCISE

IMMEDIATELY
POST-EXERCISE

FIGURE 15
DELTA FVC: ASCORBIC ACID VS PLACEBO
(MEAN \pm SEM)

FIGURE 15



FIGURE 16
DELTA FE₁: ASCORBIC ACID VS PLACEBO

(MEAN \pm SEM)

FIGURE 16

DELTA FE₁: ASCORBIC ACID VS PLACEBO

(MEAN \pm SEM)



IMMEDIATELY
POST-EXERCISE

FIVE-MINUTE
POST-EXERCISE

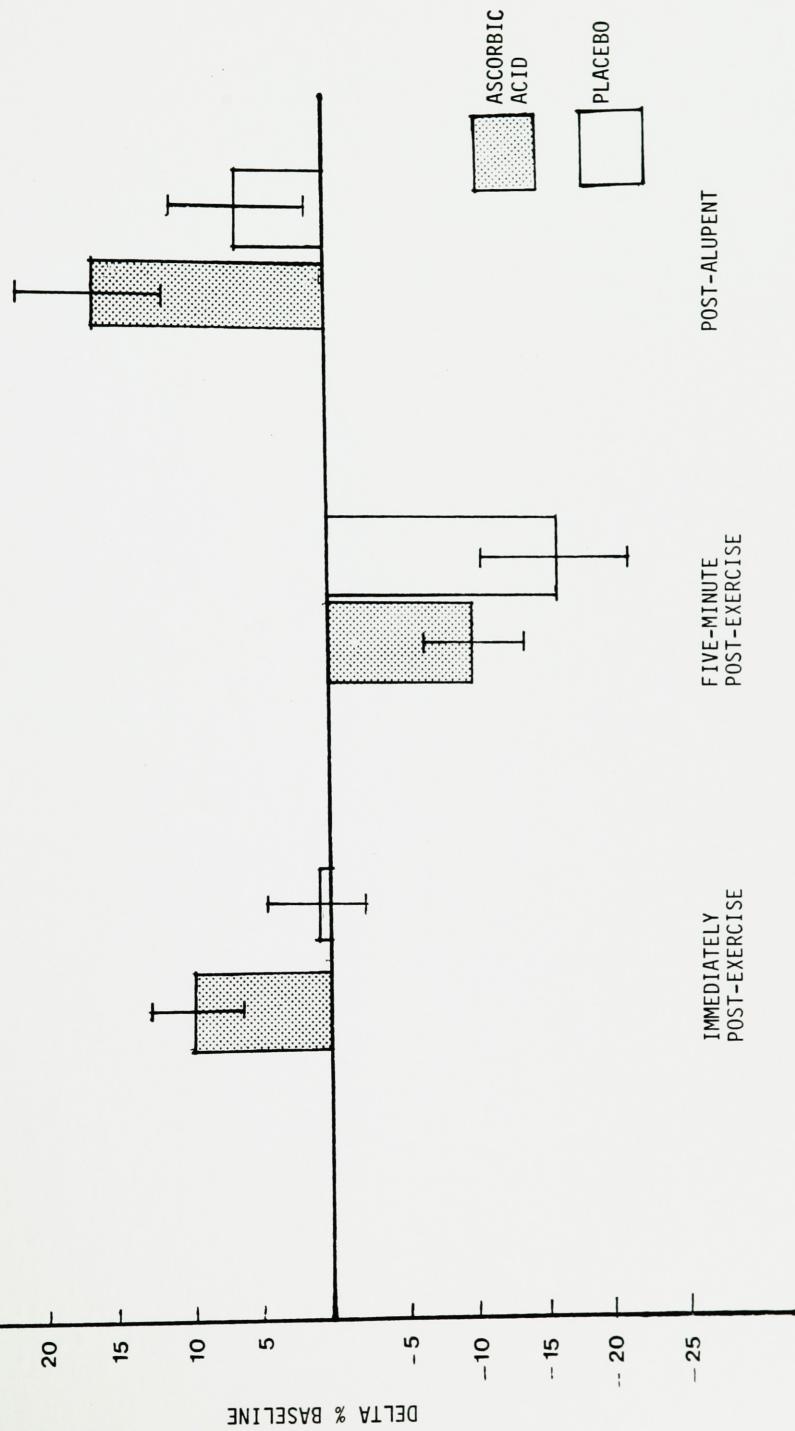
POST-ALUPENT

ASCORBIC
ACID

PLACEBO

FIGURE 17
DELTA PEFR: ASCORBIC ACID VS PLACEBO
(MEAN \pm SEM)

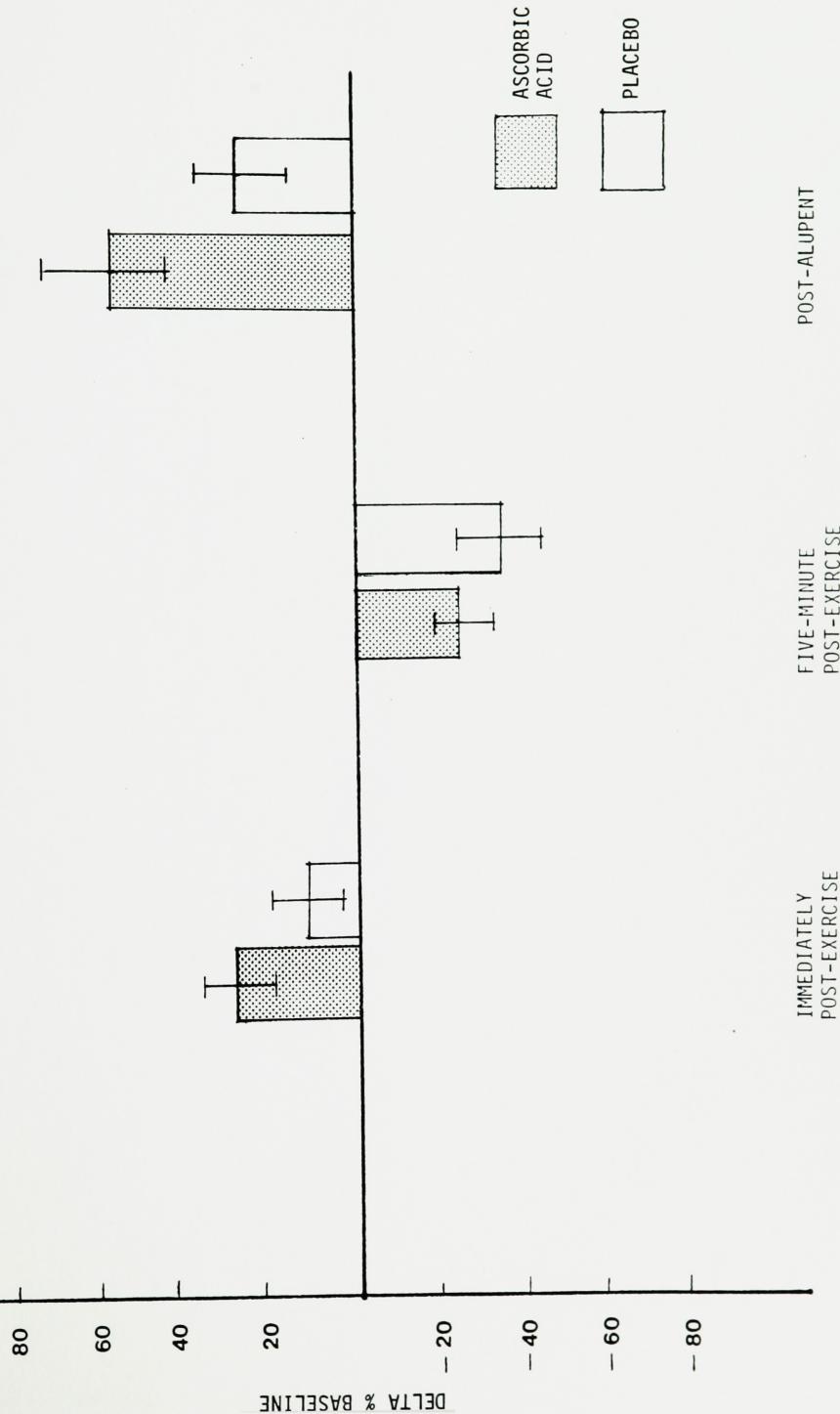
FIGURE 17



DELTA MEF40%: ASCORBIC ACID VS PLACEBO

FIGURE 18

(MEAN \pm SEM)



IMMEDIATELY
POST-EXERCISE

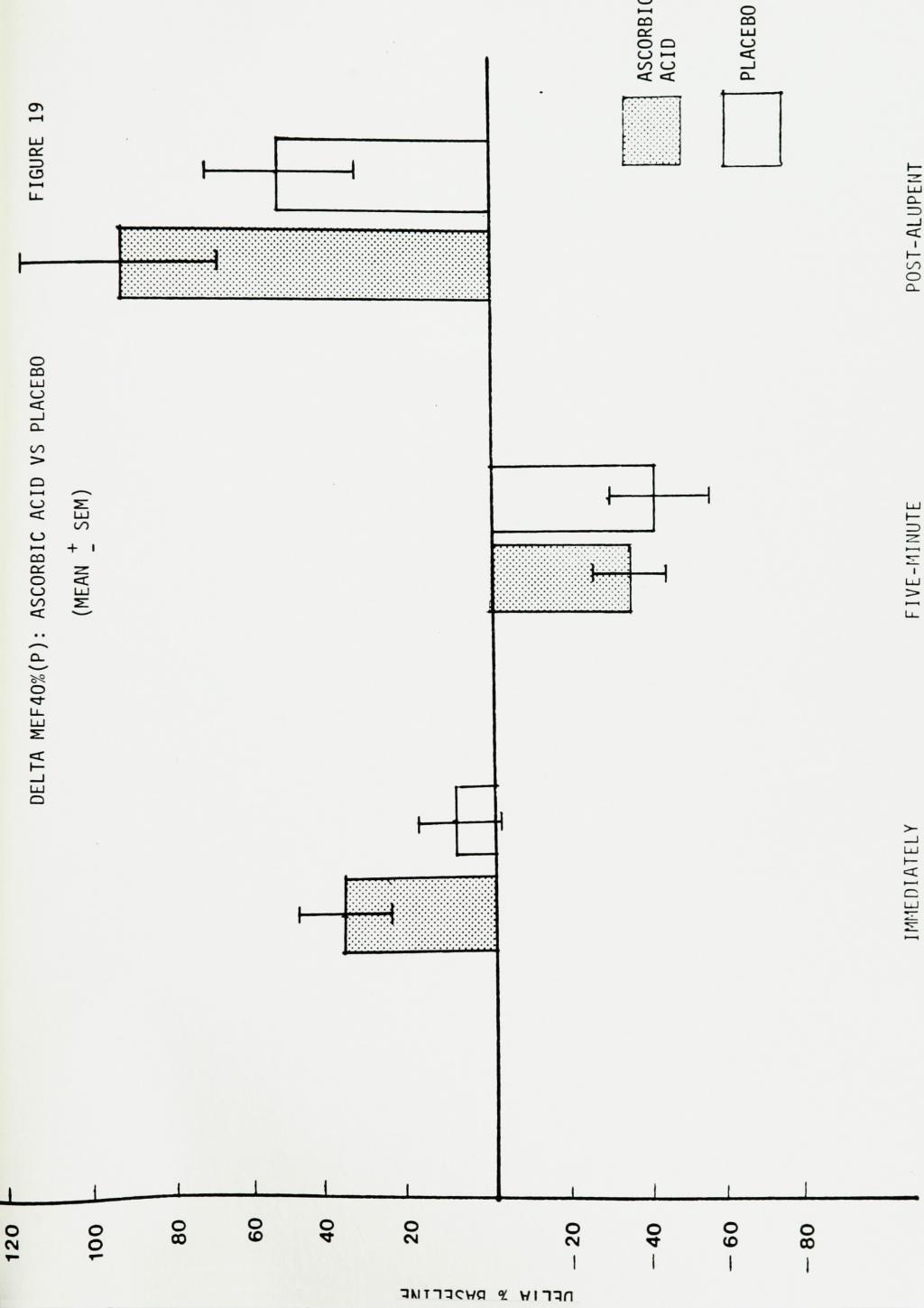
FIVE-MINUTE
POST-EXERCISE

POST-ALUPENT

FIGURE 19

DELTA MEF_{40% (P)}: ASCORBIC ACID VS PLACEBO

(MEAN \pm SEM)



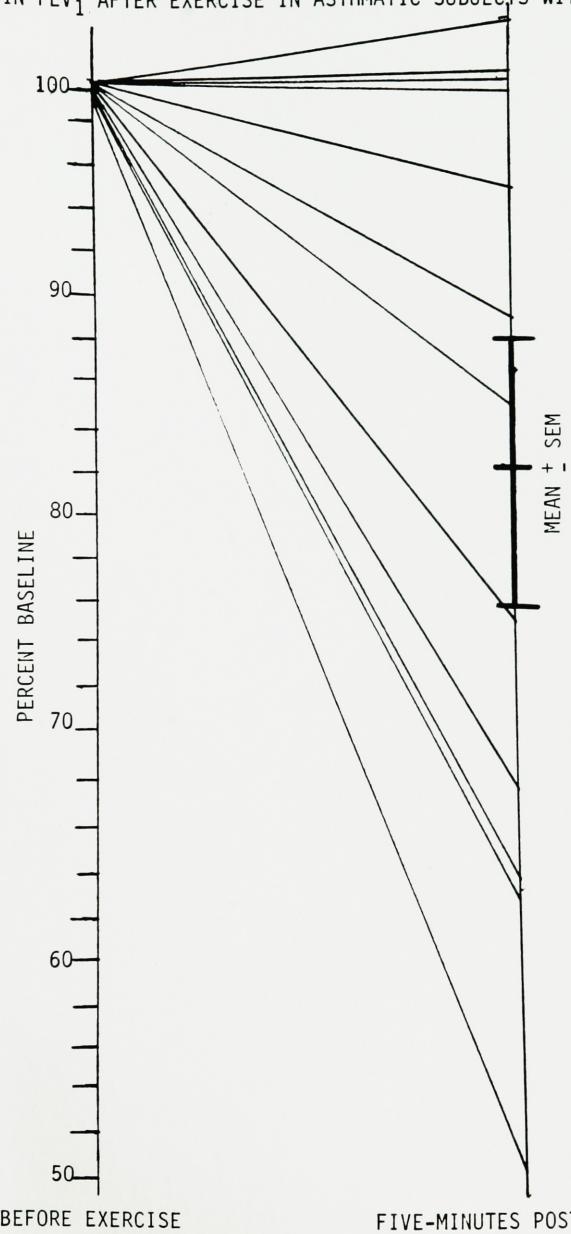
IMMEDIATELY
POST-EXERCISE

FIVE-MINUTE
POST-EXERCISE

POST-ALUPENT

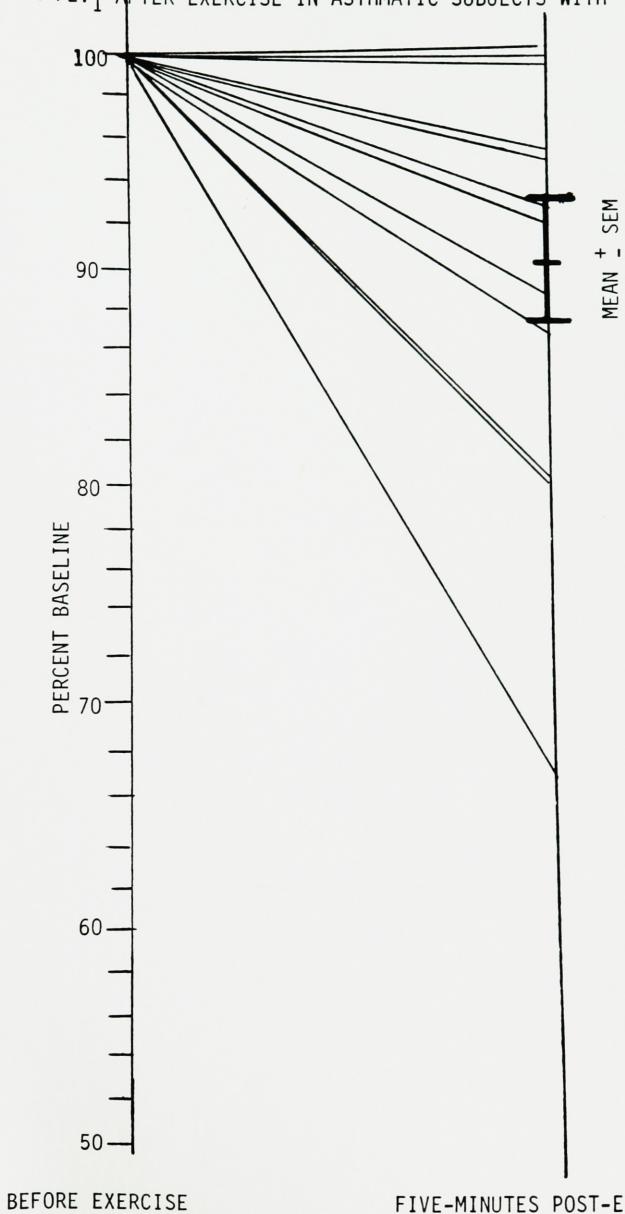
CHANGE IN FEV₁ AFTER EXERCISE IN ASTHMATIC SUBJECTS WITH PLACEBO

FIGURE 20
(PART 1)



CHANGE IN FEV₁ AFTER EXERCISE IN ASTHMATIC SUBJECTS WITH AA

FIGURE 20
(PART 2)



THEORIES FOR THE MECHANISM OF THE ATTENUATION OF EIB BY ASCORBIC ACID

FIGURE 21

— stimulation
- - - inhibition

HISTAMINE LEVELS

ALTERED OXIDATION/
REDUCTION POTENTIAL

AA

EPINEPHRINE LEVELS

PG E₂ LEVELS

b

b

c

c

c

d

d

d

ADENYLYL CYCLASE
PDE

PDE

NEUROTRANSMITTER RECEPTOR

CALCIUM PERMEABILITY

ADENYLYL CYCLASE

PDE

HISTAMINE RECEPTOR

MAST CELL

SMOOTH MUSCLE CELLS

SARCOPLASMIC RETICULUM

CALCIUM RELEASE

RETICULUM

SARCOPLASMIC

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